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Effect of Bypass on Epithelial Morphometrics and Kinetics in Experimental Colonic Inflammation

by

Junetta P. Tabor

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Abstract

The effects of colocolic bypass in continuity on acetic acid colitis in male Sprague-Dawley rats were studied by epithelial cell counts, morphometry, ^{3}H-thymidine autoradiography and mucin histochemistry.

Intracolonic administration of 10% acetic acid provoked prompt significant increases in epithelial and proliferative parameters of both transverse and descending colonic segments; peaking at week 3 and week 1 respectively, before gradually reverting to normal levels within 6 and 8 to 10 weeks. A delayed reaction in the ascending colon was characterized by insignificant increases at week 2 prior to normalization at week 4. Early changes in all 3 segments correlated with alterations in mucin composition with a predominance of sialomucins.

Bypassing the normal colon, by contrast, resulted in marked depression of these morphological and proliferative parameters in ascending and transverse colonic segments; particularly in the former. In addition, there was a correlative tendency between atrophy and increased production of sulphomucins at the expense of sialomucins.

Defunctioning the inflamed colon not only reversed the hyperproliferative, hyperplastic and other toxic changes seen in the inflamed controls but also reduced the shift towards increased sialomucin production. Futhermore, bypass negated the inflamed changes of the proximal colon with a return to normal in the transverse and descending colon

within 2 and 4 weeks respectively; 4 weeks before inflammation had ceased in the inflamed controls.

These results suggest that bowel rest protects against the acetic acid-induced inflammatory changes demonstrated in the colon of the rat.

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List of Abbreviations and Symbols

A = Column height in number of cells.

AC = Ascending colon.

AZ = Total crypt cell population (A*Z).

Z = Column count.

B = Crypt height in micrometers.

B_1 = Distance from crypt base to tip of surface epithelium.

BPCN = Bypass controls.

C = Distance from apex of crypt base to tip of surface epithelium.

D = Apical minor crypt width.

D_1 = Medial minor crypt width.

D_2 = Basal minor crypt width.

DC = Descending colon.

E = Apical major crypt width.

E_1 = Medial major crypt width.

E_2 = Basal major crypt width.

F = F ratio.

F_1 = Number of mitotic figures in longitudinal crypt.

F_1/A = Mitotic index ($100*F_1/A$).

FBMAX = Maximum height of mitotic figures.

FBMEAN = Average height of mitotic figures from crypt base.

FHMB = Relative size of proliferative zone (100*FBMAX/B).

IBD = Inflammatory bowel disease.

IFBP = Inflamed bypass.

IFCN = Inflamed controls.

i.r. = intrarectal.

J = Number of cross-sectioned crypts per unit area (frontal sections).
M = mitotic phase.
MBBF = Methylene Blue-Basic Fuchsin.
MEAND = Average minor crypt width (mean of D, D_1, D_2).
MEANE = Average major crypt width (mean of E, E_1 and E_2).
NS = Not significant.
NC = Not calculated.
PZ = Proliferative zone height (leading edge).
r = Pearson correlation coefficient.
S = DNA-synthetic phase.
SA = Sialic acid.
SD = Standard error of the mean.
TC = Transverse colon.
TPZP = Relative size of proliferative compartment (100*PZ/B).
TVF = Thiry-Vella fistula.
um = micrometers.
X = Minor diameter of cross-sectioned crypts.
Y = Major diameter of cross-sectioned crypts.

I. LITERATURE REVIEW

A. Bypass

Experimental Bypass

Colonic Bypass

The influence of experimental bypass on colonic structure and epithelial cell kinetics has been the subject of a number of animal studies (Rijke *et al.* 1979a; Terpstra *et al.* 1981; Pon *et al.* 1982; Delvaux *et al.* 1983, 1984). These investigators have invariably used as an experimental model, colostomies with or without Thiry-Vella fistulae (Tilson *et al.* 1976; Rijke *et al.* 1979a; Terpstra *et al.* 1981; Rainey *et al.* 1983; Delvaux *et al.* 1983, 1984) whereby the excluded colon is disconnected from the rest of the intestine, as opposed to a self-emptying blind isoperistaltic loop (Pon *et al.* 1982) which is distally attached to the functioning bowel.

The adaptive response of the defunctionalized colon following experimental bypass is usually one of marked hypoplasia, mucosal atrophy and hypoproliferation (Rijke *et al.* 1979a; Williamson 1978a, 1982; Terpstra *et al.* 1981; Rainey *et al.* 1983; Delvaux *et al.* 1983, 1984). Rainey and co-workers (1983), in studying the effect of fecal and bile acid diversion on colonic carcinogenesis, isolated almost the entire colon of male Sprague-Dawley rats as a Thiry-Vella fistula (TVF); drained by two stomas that were created by bringing the transected ends of proximal and distal colonic segments to the abdominal surface. They observed that the defunctioned loop underwent marked atrophy, shown by a significant reduction in the height of the mucosal crypts as well as in the lengths and weights of the TVFs. Interestingly, this adaptive response protected against colonic carcinogenesis and was partially reversed by irrigating the TVF with feces or saline.

Rijke and his associates (1979a) performed an analagous surgical procedure on male Wistars, designed to examine the effect of bypass on the epithelial cell kinetics of the defunctioned descending colonic segment. Six weeks postoperatively, they too recorded mucosal atrophy of the bypassed loop. Mucosal hypoplasia was indicated by a substantial decrease in the crypt cell population (estimated by epithelial cell counts), that is, in the number of cells per crypt column and in particular, the number of columns per crypt circumference. The results of their autoradiographic studies, however, suggested a normal cell turnover as there was no change in the life span, migration rate and labelling index of epithelial cells, as well as in the distribution of labelled cells within the crypt. This normal cell renewal that occurred despite mucosal hypoplasia was commented on in the review of Menge and

Robinson (1983).

More recently, three other studies (Terpstra *et al.* 1981; Delvaux *et al.* 1983, 1984), using transverse colostomies to isolate the distal colon in rats, have substantiated and expanded on these bypass adaptive changes. Delvaux and colleagues (1983) found that the reduction in the number of cells per crypt column earlier described in the excluded colon by Rijke *et al.* (1979a), could occur as early as three weeks postoperatively and that, contrary to these authors, hypoplasia was paralleled by suppressed epithelial cell renewal. Hypoproliferation was indicated by a significant reduction in mitotic and labelling indices in addition to mucosal DNA specific activity at 5 days or 3 weeks, and by at least one week, respectively, after diverting proximal colostomy (Terpstra *et al.* 1981; Delvaux *et al.* 1983). Mucosal DNA, RNA and protein contents, all indicators of mucosal cellularity, were further observed by Terpstra *et al.* (1981) to be reduced after fecal diversion, by approximately a third in one week and by a half in four weeks.

Another interesting, hitherto unrecorded finding by Delvaux and his associates (1983) was the significant decrease in the proliferative parameters - mitotic index, labelling index and DNA synthetic activity - but not in mucosal height, which occurred in the functioning proximal colonic segment at three weeks after surgery;

that is, two weeks later than was seen in the defunctioned colon.

In the preliminary study of Pon *et al.* (1982), examining the effect of colonic bypass on mucosal inflammation in Sprague-Dawley rats, an end-to-side ileocolostomy was used to defunction the colon. They found that following bypass, there was a substantial decrease in the number of cells per crypt column in the bypassed descending colon within two weeks of the operation and that bowel rest aided in the healing of experimentally-induced mucosal inflammation.

Small Intestinal Bypass

Unlike in the case of the colon, several small intestinal bypass studies have been carried out in the rat and although most have reported progressive mucosal hypoplasia in bypassed segments (Gleeson *et al.* 1972 a,b; Dowling and Gleeson 1973; Menge *et al.* 1974; Hanson *et al.* 1977; Rijke *et al.* 1977; Williamson 1978a, 1982; Skagen 1984), some have noted little (Fenyo and Hallberg 1976; Williamson *et al.* 1978; Williamson and Malt 1980a,b) or no change (Clark 1974; Williamson 1978a).

That adaptive hypoplasia and reduced mucosal mass have frequently been found in excluded small bowel segments, is well documented by several studies. In Thiry-Vella bypassed fistulae, total crypt cell population, crypt column and villus column were significantly reduced within one to two weeks of the operation and remained constant for up to twelve months thereafter (Hanson *et al.* 1977; Rijke *et al.* 1977). A similar marked reduction in villus height, crypt height and crypt:villus ratio was noticed in bypassed loops following enteroenteric and Thiry-Vella bypass surgery (Gleeson *et al.* 1972a; Menge *et al.* 1974; Olubuyide *et al.* 1984). Changes in villus morphology other than a reduction in height were villus broadening (Menge *et al.* 1974) and a switch from the adult pattern of transverse mucosal ridges, to a neonatal form of 'fingers' and 'leaves' (Gleeson *et al.* 1972a; Dowling and Gleeson 1973).

Additional atrophic changes observed in the excluded intestinal segments in rats, were diminished enzyme activities (Gleeson *et al.* 1972b; Skagen 1984); decreased total mucosal thickness (Menge *et al.* 1974); and reduced mucosal mass. This last finding was exemplified by a reduction in mucosal weight (Gleeson *et al.* 1972b) and in mucosal RNA, DNA and protein contents (Gleeson *et al.* 1972b; Williamson *et al.* 1978; Garrido *et al.* 1981. At the macroscopic level, the defunctionalized loop narrowed (Gleeson *et al.* 1972b; Menge *et al.* 1974; Williamson *et al.* 1978); becoming progressively thinner further and further away from the anastomotic site, where it can be quite distended (Nygaard 1967; Fenyo and Hallberg 1976; Olubuyide *et al.* 1984).

Adaptive hypoplasia may be accompanied by depressed epithelial cell renewal as is shown by decreased cell turnover (Gleeson *et al.* 1972a) and reduced tritiated thymidine incorporation in isolated whole crypts (Hanson *et al.* 1977). This, however, is not a universal finding as other studies have implied a normal cell turnover indicated by the occurrence of no change in the percentage of labelled cells and their distribution within the crypt (Rijke *et al.* 1977); the relative size of the proliferative zone of the crypt (Rijke *et al.* 1977) and the DNA specific activity (Williamson *et al.* 1978; Williamson and Malt 1980a).

Although villus height and crypt depth were usually decreased in the non-functioning segment, these parameters along with the number of crypts per length of the small bowel may be unchanged (Clark 1974; Rijke *et al.* 1977; Williamson 1978a; Williamson *et al.* 1978; Williamson and Malt 1980a), slightly decreased (Fenyo and Hallberg 1976; Williamson *et al.* 1978; Williamson and Malt 1980a,b) or even increased (Tilson and Wright 1970). These observations suggest that normal mucosal morphology can be preserved, despite macroscopic intestinal atrophy (Williamson 1978a; Williamson and Malt 1980a,b).

Clinical Bypass

Effect of Bypass on the Normal Human Bowel

The morphological adaptive response of the human small bowel to bypass is similar to that seen in experimental animals (Williamson 1978a, 1982; Hughes and Ducker 1982). In some clinical studies, jejuno-ileal bypass for severe obesity resulted in mucosal atrophy in the bypassed loop, with 30% shorter villi and shallower crypts (Fenyo *et al*. 1976; Dudrick *et al*. 1977; Asp *et al*. 1979; Miskowiak and Andersen 1983). A reduction in thymidine kinase activity further suggested mucosal hypoplasia (Stein and Wise 1978). There is, however, evidence for normoplasia from other studies in which bypassed jejunal mucosa showed no sign of atrophy, even after prolonged exclusion of three and a half and six years (Solhaug 1976; Tompkins *et al*. 1977); was histologically normal at light and ultrastructural levels (Ivey *et al*. 1979); and contained villi having an average height (336 um) similar to normal (350 um) (Daley *et al*. 1977).

Bypass in the Management of Inflammatory Bowel Disease (IBD)

Large Bowel IBD

The concept of fecal diversion to rest the bowel is not new. In 1913, Brown was the first surgeon to perform diverting ileostomy in order to provide "physiological rest" for the inflamed large bowel and this surgical procedure subsequently, became the operation of choice for certain cases of inflammatory bowel disease during the 1940's and 1950's (Homan and Dineen 1978; Goligher 1980). Since then, split ileostomy has been shown in some cases to be effective in treating patients with Crohn's colitis (Truelove *et al*. 1965; Kivel *et al*. 1967; Oberhelman *et al*. 1968; Lee 1975; Harper *et al*. 1983; Lee and Harper 1983).

In one series (Oberhelman *et al*. 1968), all thirteen patients had prompt symptomatic relief, with no evidence of recurrence or extension of their disease at a mean follow-up of 16 months. Their bowels showed evidence of healing both histologically and radiologically and three had intestinal continuity restored, one remaining well three years thereafter. These satisfactory results that were earlier substantiated in other works (Truelove *et al*. 1965; Kivel *et al*. 1967), appeared to be particularly associated with a double-barrelled ileostomy technique which allowed defunctioning of the colon to be combined with steroid irrigation (Truelove *et al*. 1965). In another recent study on a large series (Harper *et al*. 1983), describing the 20 year long-term follow-up of 102 patients who had had split ileostomy or ileocolostomy for Crohn's disease of the colon, immediate clinical remission occurred in 95 cases and was sustained in 65.

In 61 cases, ileostomies were closed, 27 of these (49%) without a minor or major resection. Promising results on restoration of bowel continuity without the need for resection, were also reported by Lee (1975) and Lee and Harper (1983).

Not all surgeons, however, have reported favourable responses to bowel rest by patients with Crohn's colitis (Jones *et al* 1966; Burman *et al*. 1971; McIlrath 1971; Young *et al*. 1975; Zelas and Jagelman 1980). In McIlrath's (1971) study, 4 of 13 patients with colostomy or ileostomy showed persistence or worsening of the disease and subsequently required proctocolectomy. Of two others who underwent colostomy closure after subsidence of the disease, one relapsed. And although patients generally showed initial improvement in their wellbeing, resolution of the symptoms and of the histological and radiological signs of inflammation seldom ever occurred. Similar ineffective results have been reported by Jones and colleagues (1966), Burman and co-workers (1971) and Young and his associates (1975).

In 1980, Zelas and Jagelman noted in their series of 79 patients with loop ileostomies, that although 72 (91%) showed early clinical improvement, 33% (28 of 72) relapsed at an average of 11 months following fecal diversion. Moreover, all three patients who had their ileostomies reconnected, also relapsed.

In contrast to Crohn's colitis, fecal diversion is ineffective in the treatment of ulcerative colitis. This is clearly shown by almost universal unsustained clinical improvement as well as by persistence and/or recurrence of the disease in the defunctioned colon (Truelove *et al.* 1965; Kivel *et al.* 1967; Oberhelman *et al.* 1968, 1976; Goligher 1980; Harper *et al.* 1983; Lee and Harper 1983). Probably the only opposing observation in the literature is that of the pioneer, Brown (1913), who after establishing an ileostomy in combination with an irrigating cecostomy, reported dramatic clinical remissions in patients with ulcerative colitis of the sigmoid colon and rectum.

Small Intestinal IBD

The results for bypass in the treatment of Crohn's disease of the small intestine parallel those for the treatment of its large bowel counterpart. On the one hand, some studies suggest that fecal diversion does not effectively alter the disease activity or course of regional enteritis (Brill *et al.* 1966; Alexander-Williams *et al.* 1972; Homan and Dineen 1978; Fazio 1983). For example, a recent series in the literature (Homan and Dineen 1978), showed a high incidence of continued or recurring disease activity in the defunctioned ileocecum. With a 15 year follow-up, these rates were 82% and 95% for ileotransversostomy bypass with exclusion or bypass in continuity (simple) respectively. Similarly, in the series of Koudahl and his associates (1974), who also employed exclusion and nonexclusion bypass procedures, the disease either persisted or recurred in or extended from the bypassed loop in 67% of cases after a mean follow-up of 2.6 years. Nonetheless, in the remaining 33% (5 of 15) there was no histological indication of inflammation in the isolated bowel after 9 years. A high rate of reoperation was documented in three separate studies (Alexander-Williams *et al*. 1972; Koudahl *et al*. 1974; Homan and Dineen 1978) and was indicated primarily for fistulae and abscess formation in the bypassed bowel.

Some studies, on the other hand, have reported satisfactory results following bowel rest, whether from anastomotic entero-colostomy or ileostomy (Garlock and Crohn 1945; Garlock *et al*. 1951; Heaton *et al* 1964; Aufses and Kreel 1971). The excellent prognosis of President Eisenhower seven and a half years after he was treated for regional enteritis by simple bypass in continuity attests to this (Heaton *et al*. 1964). In the Mount Sanai Hospital series of Garlock and colleagues (Garlock and Crohn 1945; Garlock *et al*. 1951), also, all but 9 cases (total, 65) had no recurrences two to fourteen years following side-to-end ileotransversostomy or ileosigmoidostomy. Furthermore, resolution of the inflammatory process was substantiated histologically and bypass resulted in lower recurrences than did resection.

B. Colonic Atrophy as a Result of Reduced Intraluminal Load

It has been suggested that the hypoplasia and hypoproliferation often seen in the defunctioned colon following fecal diversion results, at least in part, from the absence of intraluminal fecal bulk with a concommitant loss of mechanical stimulus that is needed for the maintainance of colonic mucosal architectural integrity and normal cell proliferation (Stragand and Hageman 1977; Ryan *et al*. 1979; Williamson 1982; Menge and Robinson 1983; Delvaux *et al*. 1983, 1984). This suggestion is based on the results of several experiments on dietary manipulations such as starvation (Hagemann and Stragand 1977; Stragand and Hagemann 1977; Delvaux *et al*. 1984; Majumdar 1984), parenteral nutrition (Ryan *et al*. 1979; Morin *et al*. 1980) and chemically defined diets (Janne *et al*. 1977; Ryan *et al*. 1979; Morin *et al*. 1980; Storme and Willems 1981), which are known to reduce fecal bulk in the colonic lumen.

The adaptive response of the colon to the cessation of bulk food and oral intake is well documented. Chemically-defined elemental diets, which are almost totally absorbed at the jejunal level (Morgan *et al*. 1970; Menge and Robinson 1983), thus providing little or no residue in the large bowel, mimic the atrophic changes promoted by experimental bypass. Four separate studies (Janne *et al*. 1977; Ryan *et al*. 1979; Morin *et al*. 1980; Storme and

Willems 1981), have shown that in elemental fed rats, the colon atrophies within 6 days to 4 weeks, showing significant decreases in crypt column length, crypt column count, total crypt cell population, colonic weight, mucosal DNA and protein contents, DNA synthesis, and mitotic and labelling indices. These changes are associated with extended DNA synthesis phase, cell cycle time and turnover time. In two of these works, column length, column count, the number of cells per crypt and colonic weight were reduced, respectively, by 49%, 45.5%, 75% and 56% within 4 weeks of the diet (Janne *et al*. 1977) and the proliferative zone of the crypts was shortened after only 6 days (Storme and Willems).

Parenteral nutrition in rats induces similar changes in the colon (Ryan *et al*. 1979; Morin *et al*. 1980). Jannne *et al*. (1979) and Storme and Willems (1981) have suggested that mucosal atrophy is a consequence of decreased epithelial cell proliferation. Hypoproliferation in turn, is attributed to a lack of bulk in the colonic lumen, as supplementing a liquid diet with non-absorbable bulk restores colonic weight and DNA synthetic activity, independent of the activity of the hormone, gastrin (Ryan *et al*. 1979).

The dependence of colonic integrity and cell proliferation on the presence of luminal load is further indicated by the results of fast-feeding experiments. Fasting in rodents immediately induces hypoproliferation, characterized by shortening of the proliferative zone

(Wiebecke *et al*. 1969) as well as by reduction in thymidine kinase activity and tritiated thymidine incorporation (Wiebecke *et al*. 1969; Hagemann and Stragand 1977; Majumdar 1984). These changes are accompanied by prolongation of the G1 and S phases and consequently, of the cell renewal cycle (Wiebecke *et al*. 1969; Hagemann and Stragand 1977; Eastwood 1977; Menge and Robinson 1983).

Refeeding starved rodents with standard chow pellets, but not a low residue diet, promptly reverses the fasting response and results in hyperproliferation (Hagemann and Stragand 1977; Majumdar 1984). This postprandial rise in cell proliferation occurs long before increases in serum and tissue gastrin levels (Majumdar 1984). Moreover, the refeeding response is diminished in distal colonic segments when the ascending colon is ligated (Stragand and Hagemann 1977). Thus, postprandial hyperproliferation has been ascribed to the entrance or presence of bulk in the colonic lumen (Hagemann and Stragand 1977; Stragand and Hagemann 1977). However, Delvaux and his co-workers (1984) have recently shown that intraluminal postprandial factors are major, but not the only ones involved in the regulation of colonic epithelial cell proliferation, since refeeding starved rat which had transverse colostomies, increases cell proliferation in both excluded distal and proximal functioning colonic segments.

C. Acetic Acid Colitis

In rats, a readily reproducible, acute, diffuse, chemical colitis that resembles the human disease both grossly and histologically, can be induced by intraluminal administration of various amounts of 5 to 10% acetic acid. This experimental model was initially developed by MacPherson and Pfeiffer (1976, 1978) and has since then been used by several others (Mann *et al.* 1979, 1980; Hagihara 1982; Mann and Demers 1983; Sharon and Stenson 1985).

Mucosal contact with 10% acetic acid for 10 to 15 seconds results in inflammation without perforation (MacPherson and Pfeiffer 1978; Mann *et al.* 1980). Characteristically, the colitis induced is restricted to the distal colon (MacPherson and Pfeiffer 1978). However, it can extend for variable distances from the rectum to the ascending colon (Hagihara 1982) and is primarily localized to the mucosa and submucosa although it can involve all four layers (MacPherson and Pfeiffer 1978).

Histologically, the major features are as follows: mucosal and submucosal edema; vascular congestion; pseudopolyp and ulcer formation; coagulative necrosis; decreased goblet cell mucus; cryptitis; increased submucosal fibrosis; crypt hypertrophy and hyperplasia; and increased polymorphonuclear neutrophilic leukocyte infiltration of the mucosa and submucosa.

The evolution and progression of acetic acid colitis, evaluated by fiberoptic colonoscopy and biopsy, was described by Mann *et al.* (1979, 1980) and Mann and Demers (1983). Hyperemia was observed within 10 hours of 1.5 ml of 10% acetic acid. Ulcers were evident at 13 hours, became more pronounced at 24, and were immersed in a yellowish exudate by 3 days. Submucosal fibrosis and epithelial regeneration were seen at 24 days . By 8 weeks finally, the lesions had healed both endoscopically and histologically. In slight contrast, 0.5 ml of 10% acetic acid produced ulcers within 3 days (MacPherson and Pfeiffer (1976).

The mechanism (s) by which acetic acid induces colonic inflammation and mucosal injury is poorly understood. Prostaglandins may be involved as intracolonic administration of indomethacin is associated with lower levels of protaglandin E_2 in acetic acid-treated mucosa and with reduced inflammatory activity (Mann *et al.* 1983). Moreover, it has recently been shown (Sharon and Stenson 1985) that compared to normal, acetic acid-treated mucosa converted more exogenous arachidonic acid to Leukotriene B_4 (LTB_4) and 5-hydroxyeicostetraenoic acid (5-HETE).

Arachidonate metabolites appear to be produced primarily by neutrophils both in experimental colitis and inflammatory bowel disease (Sharon and Stenson 1984, 1985) and neutrophil infiltration is characteristic of this animal model (Mann and Demers 1983). Leukotriene B_4 which induces vascular permeability (Bjork *et al.* 1982), might therefore play a role in the mucosal and submucosal edema and diarrhea which are common features of this rat model.

The ulcerogenic effect of this acid might be related to increased fibrinolytic activity. This appears to be one mechanism by which bloody diarrhea and rectal bleeding are induced in the rat model (Mann *et al.* 1979, 1980). In support of this is the observation that epsilon-aminocaproic acid (E.A.C.A.), an anti-fibrinolytic agent, protects against acetic acid colitis by reducing the enhanced fibrinolytic activity associated with it (Mann *et al.* 1979, 1980) and with human active ulcerative colitis (Kwaan *et al.* 1969). The clinical use of E.A.C.A. also promptly relieved rectal bleeding in a considerable number of ulcerative colitic patients (Salter and Read 1970).

Acetic acid colitis shares some of the histological features of ulcerative colitis. These include crypt abcesses, mucosal and submucosal edema, ulcer and pseudopolyp formation and neutrophil infiltration (MacPherson and Pfeiffer 1978; Mann *et al.* 1983; Nixon and Ridell 1983; Sharon and Stenson 1985). Colonic involvement is diffuse and is usually limited to the mucosa and submucosa. Differences exist, however; inflammatory bowel disease is chronic with exacerbations and remissions (Nixon and Ridell 1983), whereas the rat model is acute and resolves fairly rapidly (Mann *et al.* 1980).

D. Colon Morphology

Gross Morphology

The large bowel of the white rat (Wistar/Furth) whose total length is about 21 to 27 cm in the adult, comprises five parts: a large cecum, 5 to 7 cm long; the ascending or proximal colon, 5.5 to 6.5 cm long; the transverse colon or major flexure, 3 to 3.5 cm in length; the descending or distal colon, 4.5 to 6.5 in length; and the rectum (Lindstrom *et al.* 1979). The colon of the rat, unlike that of man, has no haustra. These are presumably replaced by numerous mucosal folds which exhibit segmental variations (Lindstrom *et al.* 1979; Shamsuddin and Trump 1981; Menge and Robinson 1983). The ascending colon is characterized by oblique mucosal folds with a "herringbone" or "palm-leaf" arrangement whereas both the transverse and descending colon have a pattern of longitudinal folds.

Microanatomy

The colon, like the rest of the intestine, consists of four layers, namely, mucosa, submucosa, muscularis externa and serosa (Lindstrom *et al.* 1979; Shamsuddin and Trump 1981; Krause and Cutts 1984). The mucosa, which lines the colonic lumen, is further divisible into a simple columnar epithelium, a lamina propria and a muscularis mucosae. The single cell thick epithelium which lines numerous, closely packed, unicellular glands, the crypts of Lieberkuhn and the flat mucosal surface, may consists of five cell types in rodents - vacuolated, columnar or absorptive, mucous, enteroendocrine or argentaffin, and caveolated (Chang and Leblond 1971; Chang and Nadler 1975; Tsubouchi and Leblond 1979; Tsubouchi 1981; Shamsuddin and Trump 1981).

Columnar absorptive cells are predominant in the upper third of the crypt and on the surface (Chang and Leblond 1971; Tsubouchi 1981; Shamsuddin and Trump 1981; Thomopoulos *et al*. 1983). They originate from immature, multipotential, crypt-base stem cells, differentiate while moving up the crypt to the surface, and are ultimately extruded into the colonic lumen (Chang and Leblond 1971; Eastwood 1977; Lipkin 1981; Menge and Robinson 1983). They turn over in 2 to 3 days in rodents and 3 to 8 days in human beings (Messier and Leblond 1960; Cole and McKalen 1961; Lipkin *et al*. 1963; MacDonald *et al*. 1964; Shorter *et al*. 1964; Chang and Leblond 1971; Eastwood 1977; Lipkin 1981; Eastwood 1983).

Mucous cells arise from the same undifferentiated, crypt-base vacuolated cell as the columnar cell with which they migrate, and from the mitosis of immature, "oligomucous" cells, found in the lower half of the crypt (Chang and Leblond 1971; Porcella *et al*. 1979) They are distributed throughout the crypts and the surface epithelium. Two mature, non-mitotic mucous cells have been described in the colon of rodents and man (Shamsuddin and Trump 1981; Altmann 1883; Thomopoulos *et al*. 1983; Altmann and Frei 1984). The goblet or large-granuled Type II cell is localized at the upper crypt and surface epithelium whereas the "deep crypt secretory" or small-granuled Type I cell, with abundant mucus globules, is found exclusively in the lower half of the crypt.

In the rat, "deep crypt secretory cells" comprise, respectively, 33%, 21% and 5% of the crypt cell population of ascending, transverse and descending colonic segments (Altmann 1983). While Type I cells are abundant in the ascending colon, Type II goblet cells appear to increase in number toward the distal colon, constituting 20 to 30% and 40 to 50% of crypt epithelium, respectively, in ascending and descending colonic segments in man.

According to two recent studies on the rat (Shamsuddin and Trump 1981; Altmann 1983), more mucous-containing cells are found in the lower half of the crypt than in its upper portion, in the ascending colon; the reverse is true of the descending colon, despite the report of another study that the deep crypt epithelium of rodent rectosigmoid colon consists primarily of mucus-secreting cells (Thomopoulos *et al*. 1983). One early report (Martin 1961), however, claims that there are more mucous cells in the lower half of the crypt, irrespective of colonic segment.

Briefly, the other three cell types are: the vacuolated cells, poorly differentiated stem cells believed to give rise to all other types; enteroendocrine cells; and caveolated cells (Chang and Leblond 1971; Tsubouchi and Leblond 1979; Chopra *et al*. 1981; Shamsuddin and Trump

1981). The first of these is localized at the lower two-thirds of the crypt, whereas the other two are scattered throughout the mucosal epithelium. In rodents, enteroendocrine and caveolated cells turn over in 23.2 and 8.2 days respectively, the latter migrating jointly with columnar and mucous cells.

E. Normal Crypt Morphology

The shape and size of the crypts of Lieberkuhn differ from region to region along the length of the rodent colon. For example, in the ascending colon of Wistar rats (unlike F344), the crypts are basally branched (Filipe 1975; Lindstrom *et al*. 1979; Shamsuddin and Trump 1981). But in the transverse and descending colonic segments of both types of animals, they are straight and tubular. Basal bifurcation is also characteristic of the proximal colon of BD IX rats, whether 4 days or 6 months old (Maskens and Dujardin Loits 1981).

Cytokinetic studies on Wistar rats (Sunter *et al*. 1978, 1979) have shown that the total number of cells within the crypt is similar in the descending and transverse colon, with 735 cells but lower in the ascending colon, with 630 cells, and higher in the cecum, with 760 cells. The data suggest that there are slight segmental differences in the absolute size of the crypts. Corresponding mean column count values, that is, the number of cells per crypt circumference, were 17 to 17.6, 19 and 23 cells. The mean number of cells per crypt column of the descending and transverse colon was 43 cells, while that of the other two regions was 33 cells, indicating that crypts in the distal segments are similar in length and longer than those of the proximal colon.

The proximal-to-distal increase in colonic crypt length estimated by cell counts, was further substantiated morphometrically by Gutschmidt *et al.* (1983); in the cecum, ascending colon, descending colon and rectum, crypt lengths were estimated as 147.1, 156.3, 163.3 and 169.3 micrometers (um), respectively. In Wistar/Furth rats, by contrast, compared to other colonic segments, the longest crypts were found in the transverse colon (Lindstrom *et al.* 1979). Furthermore, in Sprague-Dawley rats, crypts of both the ascending and descending colon appear to be similar in length with approximately 40 cells per crypt column (Tilson and Livstone 1980).

Compared to old Wistars, the descending colon of younger animals, have a lower number of cells per crypt, in the order of 625 cells; the mean column count and crypt length values being 19 and 33 cells respectively. (Rijke *et al.* 1979b). That the mean number of cells per crypt varies with the age of the animal, was reported by Maskens and Dujardin-Loits (1981), who observed that total crypt cell population rose from 249 cells in 4-day-old rats to 653 cells in adults.

Male Sprague-Dawley rats, on the other hand, are reported to have a mean crypt length of 38.2 or 40 cells in the descending colon, similar to that of adult Wistars (Tutton and Barkla 1976; Tilson and Livestone 1980). These two strains, however, show a considerable difference in their mean column counts; the former having a circumference more than twice that of the latter (39.5 cells as opposed to 17.6 cells) (Tutton and Barkla 1976; Sunter *et al*. 1979). In younger male Sprague-Dawleys, the mean height of the crypt was estimated as 32 cells (Heitman *et al*. 1983).

In the mouse, mean crypt length has been variously described as 20.3 cells in the ascending and sigmoid colon by Lipkin and Quastler (1962), and as 29 and 31.1 cells in the descending colon by Chang and Leblond (1971) and de Rodriquez *et al*. (1979). A column count of 22.5 cells observed by the latter investigators, contributed to a crypt cell population of 700 cells, that was later confirmed in a more detailed cytokinetic study of the entire colon (Appleton *et al* 1980). In this study also, the total crypt cell population in the ascending colon (310 cells) was found to be half that of the other three colonic segments (average of 670 cells); differing somewhat from observations made in a similar study of the rat (Sunter *et al*. 1979). In addition, as was seen in Wistar rats, the distal two colonic segments contained crypts that were similar in length but longer than those of other regions.

F. Proliferation of Normal Colonic Epithelial Cells

The epithelium of the gastrointestinal tract is in a steady-state of cell renewal, with the rate of cell production (mitosis) in the crypt balancing cell loss at the surface (Lipkin and Deschner 1976; Eastwood 1977). The epithelial renewal system of the colon is compartmentalized and as such, proliferating and maturing cells are localized in the crypts while functional cells are found within the crypt and on the surface (Wright 1980).

In the rodent colon, cell proliferation is restricted to the lower two-thirds of the crypt (de Rodriquez *et al.* 1979; Sunter *et al.* 1979; Barkla and Tutton 1983). The distribution of the proliferating cells within this region, however, varies according to colonic segment. Cytokinetic studies in the rat (Tutton and Barkla 1976; Sunter *et al.* 1978, 1979; Rijke *et al.* 1979b), reveal that in the descending colon, the greatest number of tritiated thymidine labelled cells are confined to the bottom of the crypt. In ascending and transverse colonic segments, though, most proliferating cells are found in the middle of the gland whereas in the cecum, a higher number of labelled cells are again localized to the basal crypt. In addition, the most basally located cells of the last three sites undergo little proliferative activity indicated by their low labelling indices. A similar distribution pattern exists in the colon of mice except that, maximum label in the transverse colon occurs at a cryptal level intermediate to those of ascending and descending colonic regions (de Rodriquez *et al*. 1979; Appleton *et al*. 1980).

Apart from distribution, segmental variation exists in the amount of proliferating cells present in the proliferative compartment. According to Sunter *et al*.'s (1979) kinetic study, the ascending colon of of rats has a lower growth fraction (0.23) and thus, a lower number of proliferating cells (145), than all other colonic segments.

During the cell cycle, undifferentiated cells go through four successive phases; two of these being the mitotic phase (M) and the DNA-synthetic phase (S). At any one time, there are approximately 15 to 20 times more S phase cells than mitotic figures in the poliferative zone (Willems 1979), the former comprising 12 to 14% of the colonic epithelial cell population in the mouse (Cheng and Bjerknes 1982).

Duration of the cell cycle and its phases, estimated by the fraction of labelled mitoses (FLM) method, differs according to colonic site. Generally speaking, however, the complete cell cycle last 25 to 58 h in rats (Sunter *et al*. 1978, 1979; Rijke *et al*. 1979b) with S values ranging from 7.6 to 9 h. Mitosis itself, is very brief and last about 1 h (Eastwood 1973).

G. Proliferation Kinetics of IBD

Clinically and experimentally, inflammation of the colon is associated with hyperplasia, enhanced cell proliferation and an abnormal proliferative pattern (Watt and Marcus 1970; Eastwood and Trier 1973; Serafini *et al.* 1981; Pon *et al.* 1982). Abnormal cell proliferation is in one way exemplified, by unusually high mitotic activity and by the presence of mitotic figures in the upper third of the crypts and on the surface of dysplastic mucosa, during the precancerous phase of ulcerative colitis (Butt *et al.* 1983).

Studies on *in vitro* labelling of ulcerative colitic rectal biopsies, have also shown an expansion of the proliferative zone of the crypt toward the luminal surface, along with accelerated cell migration and shorter turnover time (Bleigberg *et al.* 1970; Eastwood and Trier 1973). The number of tritiated thymidine labelled cells, moreover, was more than twice that of control crypts (Eastwood and Trier 1973). This increase in labelling index is also characteristic of other gastrointestinal inflammatory diseases, endoscopic duodenitis and its associated duodenal ulcers, for example (Gorelick *et al.* 1983; Bransom *et al.* 1984).

Biasco *et al.* (1979), not only confirmed these previous findings, but also found that increased tritiated thymidine incorporation in rectal biopsies and shorter turnover time correlated with the severity of ulcerative colitis. The increase in labelling indices described in these studies, was greater in patients with severe colitis than in those with a slight form of the disease. In turn, patients with slight ulcerative colitis showed a higher number of labelled cells than did those within the quiescent phase. Similarly, the mucosal turnover seen in severe ulcerative colitis was greater (that is, a shorter turnover time) than in the slight phase, which in turn, was more enhanced that that observed in colitis in remission, which was similar to normal.

In partial disagreement with the observations of Biasco *et al.*, Serafini *et al.* (1981), more recently, demonstrated that the mucosa of patients in the quiescent phase of ulcerative colitis, show an increase in labelling index similar to that seen in the active disease. Additionally, crypts from histologically normal quiescent mucosa contained proliferative cells in the upper third, the zone of non-proliferation. They suggested that this abnormal proliferative pattern reflected an increased cell proliferation, was characteristic of regenerating mucosa and was not necessarily limited to precancerous mucosa.

H. Characteristics of Colonic Mucins

Mucins of the Normal Colon

Colonic mucins which are lubricative and protective in function, are synthesized and stored in the goblet (mucous) cells until they are secreted. Mucins are high molecular weight glycoproteins which basically, consists of a protein core attached to several carbohydrate side chains (Allen 1978; Filipe 1979; Allen 1983). Two radicals, O-sulphate ester and sialic acid are present in the side chains of acid mucins. The sialic acid (N-acetyl neuramic acid) sugar itself, may or may not contain several O-acyl substituents in its polyhydroxy side chain and, as such, be substituted or unsubstituted (Reid *et al.* 1977, 1984a,c).

Histochemically, the distinction between sulphate-containing mucins and sialic acid-containing mucins is achieved with various cationic dye sequences. For example, the high iron diamine - alcian blue (HID-AB) method (Spicer 1965) stains sulphomucins brown black and sialomucins blue (Filipe and Branfoot 1976). Alternatively, the saponification - alcian blue - periodic acid - phenylhydrazine - Schiff (KOH/AB1.0/PAPS) method (Reid *et al.* 1985) differentially stains sulphomucins blue and sialomucins red.

Acid mucins, that is, sialomucins and sulphomucins, are found mixed with neutral mucins in normal colonic epithelium. Various histochemical studies have shown that the extent of sialylation and sulphation of glycoprotein carbohydrate side chains, depend on the location of their goblet cells; that is, whether they are found in the surface epithelium, various levels of the crypts, or different regions of the colon (Filipe and Branfoot 1976; Filipe 1979; Franzin *et al.* 1983; Reid *et al.* 1984a).

In the left colon of humans, sulphomucins predominate in the lower two-thirds of the crypt, whereas a variable proportion of sialomucins and sulphomucins is present in the upper crypt and surface epithelium; in different cells or together in the same cell. Conversely, in the right colon, sialomucins are present in the lower third of the crypt, while sulphomucins predominate in the upper two-thirds. An absolute prevalence of sulphomucins, however, is found in the normal colon. A moderate amount of neutral mucins, on the other hand, is characterictic of the upper crypt and surface epithelium, with an even smaller amount in the lower crypt (Filipe and Branfoot 1976; Filipe 1979).

As in human beings, the colon of the rat shows regional differences in the composition and distribution of goblet cell mucins; with striking histochemical similarities existing between the distal colon of the rat and the human colon (Filipe 1975; Thomopoulos *et al*. 1983). A mucin pattern similar to man's is described in the distal colon of female Wistars (Filipe 1975) and in the rectosigmoid colon of male Sprague-Dawley rats (Thomopoulos *et al*. 1983); sulphomucins predominate in the lower two-thirds of the crypts and sialomucins occupy the upper third. In the proximal colon of Wistars, though, there is a prevalence of sialomucins throughout the crypt, differing somewhat from the human pattern (Filipe 1975).

A slightly different pattern of mucin distribution is seen in male inbred Fischer 344 rats (Shamsuddin and Trump

1981). In the descending colon, most if not all goblet cells of the crypts, contain sulphomucins exclusively or predominantly, even though neutral mucopolysaccharides are occasionally found in crypt-base mucous cells. Yet, the mucin pattern seen in the ascending colon more closely resembles that of man in that, an abundance of sialomucins is seen in the lower one-third of the crypt, while sulphomucins and/or neutral mucins are observed in the upper crypt.

In addition to segmental and crypt level differences in the distribution of sulphomucins and sialomucins, the latter acid mucin exhibits variations in its substitution pattern. For instance, in the rat, there are more sialic acid substituents in the upper half of the colon as compared to the lower region (Reid *et al*. 1975, 1977). Nonetheless, throughout the colon, more O-acyl substituents are found in the upper half of the crypts (Al-Suhail *et al*. 1984).

Mucin Changes in Inflammatory Bowel Disease

Disease associated alterations in the quantity and distribution of epithelial mucins, as well as in the sialylation and sulphation of their carbohydrate side chains, have been known to occur in ulcerative colitis and Crohn's disease. This has been proven histochemically (Lev and Spicer 1965; Greco *et al*. 1967; Hellstrom and Fischer 1967; Filipe 1969; Filipe and Dawson 1970; Filipe and Branfoot 1976; Culling *et al*. 1979; Filipe 1979; Ehsanullah *et al*. 1982; Franzin *et al*. 1983; Al-Suhail *et al*. 1984; Reid *et al*. 1984a) and chemically (Teague *et al*. 1973; Fraser and Clamp 1975; Clamp and Fraser 1981; Podolsky and Isselbacher 1983).

Despite the conflicting views of some early studies (Lev and Spicer 1965; Greco *et al*. 1967; Hellstrom and Fischer 1967; Filipe 1969; Filipe and Dawson 1970), it is generally held that epithelial mucins, sulphomucins in particular, are reduced in ulcerative colitis, while they are preserved in Crohn's disease as is reviewed by Filipe and Branfoot (1976) and Filipe (1979). Quantitative changes in colonic mucins are apparently related to the disease index; more reduction occurring in chronic ulcerative colitis than in the inactive phase of the disease (Hellstrom and Fischer 1967; Filipe 1969; Filipe and Dawson 1970; Franzin *et al*. 1983) particularly in areas showing ulceration (ulcer margins especially) and polypoid hyperplasia (Hellstrom and Fischer 1967; Greco *et al*. 1967; Al-Suhail *et al*. 1984).

Lev and Spicer (1965) and Greco *et al*. (1967), were among the first to note histochemical changes in epithelial mucins during inflammatory bowel disease and reported an increase in sulphomucins. Filipe (1969) and Filipe and Dawson (1970), however, were the first to observe an increase in sialomucins at the expense of sulphomucins in ulcerative colitis. With the advent of cationic dye sequences for the evaluation of the relative proportions of sialomucins and sulphomucins, it became evident that the loss of sulphomucins more than that of sialomucins, contributed to the overall reduction of mucins seen in earlier studies (Filipe and Branfoot 1976; Filipe 1976, 1979; Ehsanullah *et al*. 1982; Franzin *et al*. 1983).

The results of these histochemical studies have shown that whereas the normal adult colonic mucosa contains a predominance of sulphomucins, specimens from patients with chronic ulcerative colitis exhibit an absolute excess of sialomucins, especially those showing epithelial dysplasia and "transitional" mucosa associated with colonic carcinoma and mucosal regeneration (Filipe 1979; Ehsanullah *et al*. 1982; Franzin *et al*. 1983; Habib *et al*. 1984). Alternatively, biopsies taken from patients within the quiescent phase of the disease, generally display normal mucin production with a predominace of sulphomucins; even though Ehsanullah *et al* (1982) reported sialomucin dominance during colitis in remission for cases with residual dysplasia. The change in the relative proportions of the two acid mucins appears to be related not only to the activity of ulcerative colitis, but also to its extent and duration (Ehsanullah *et al*. 1982).

Unlike colitis, Crohn's colitic mucosal specimens frequently demonstrate an abundance of sulphomucins, similar to normal (Filipe and Branfoot 1976; Filipe 1979; Franzin *et al*. 1983) rather than a predominance of sialomucins claimed by one study (Ehsanullah *et al*. 1982).

Qualitative changes in mucin production are accompanied by alterations in the pattern of mucin distribution. While in the normal descending colon sulphomucins-containing mucous cells are localized to the lower two-thirds of the crypt with sialomucin-containing cells in its upper third, in ulcerative colitis, cells containing both acid mucins, either together or separately, can be found scattered irregularly throughout the crypt (Franzin *et al.* 1983). A similar abnormal pattern in the distribution of neutral mucins is demonstrated in inactive ulcerative colitis by Greco *et al.* (1967) and seems to be related to the disease index.

Cases with "slight histological change", show a significant increase in the number of goblet cells containing neutral mucins, specfically in the sigmoid colon. By contrast, in cases with "severe histological change", that is, crypt abscess and ulceration, the goblet cells contain weakly acid mucins and are drastically reduced in number or lost. Futhermore, mucous cell hyperplasia which appears to be characteristic of quiescent ulcerative colitis, is at times associated with an increase in goblet cell size, as was reported by Franzin and his co-workers (1983).

Apart from alterations in the amount of mucin and the ratios of sulphomucins and sialomucins, further histochemical characterization of the latter, supported by chemical studies, have indicated that qualitative changes in sialic acid derivatives also occur in inflammatory bowel disease. Evidence for this are provided by the following studies which show that mucins from ulcerative colitis and Crohn's disease contain relatively less O-acyl substituted sialic acids than mucins from controls. Using the PBT/KOH/PAS and PAT/KOH/PAS histochemical techniques, Culling *et al*. (1979, 1981) demonstrated that, compared to normal, epithelial glycoproteins of ulcerative colitic patients contain a reduced amount of sialic acids substituted at the polyhydroxy side chain and that in addition, this deficit correlated with the severity of inflammation. In 1984, these findings were confirmed by Reid *et al*. in humans and by Al-Suhail *et al*. in experimental animals.

Crohn's colitis shows a similar reduction in side chain O-acyl sialic acids which is nevertheless, less marked than in ulcerative colitis (Culling *et al*. 1979, 1981). The decrease in Crohn's colitic sialic acid variants is consistent with findings of recent histochemical studies on inactive Crohn's disease of the ileum (Reid *et al*. 1984b), but is in direct contrast with the results of studies which indicate that increases in O-acyl substituents are associated with Crohn's disease of the ileum (Culling *et al*. 1977), even in those cases with histologically normal uninvolved mucosa (Reid *et al*. 1984b).

The results seen in human ulcerative colitis are substantiated by studies on degraded carrageenan-induced colitis of the large bowel of New Zealand white rabbits (Al-Suhail *et al*. 1984). Both histochemical and chemical data indicate that in the cecum as well as in the upper and lower colon, ulceration is associated with a significant reduction in the quantity of substituted sialic acids. Moreover, the histochemical data reveal that whereas substituents are severely decreased or absent at ulcer margins, they are present in areas with no ulceration.

II. INTRODUCTION

Split ileostomy is sometimes recommended as the surgical treatment of inflammatory bowel disease (IBD) unresponsive to medical treatment such as Crohn's disease of the colon. Indications for the use of bypass surgery include: as a first stage procedure for extremely ill and debilitated patients (Attisha 1982, Lee and Harper 1983); to facilitate technically difficult major and minor resections (Homan and Dineen 1978; Beart 1983; Harper *et al.* 1983); to achieve healing of the inflamed bowel, with subsequent restoration of bowel continuity (Lee 1975, 1976; Oberhelman 1976; Harper *et al.* 1983; Lee and Harper 1983); for the treatment of obstructive inflammatory masses of Crohn's disease of the duodenum and terminal ileum (Colcock 1973; Goligher 1980; Beart, McIlrath and Kelly 1980; Attisha 1982; Johnson *et al.* 1982); and as an adjunct to medical therapy, in an attempt to conserve bowel (Lee and Harper 1983).

The object of bypass surgery is to divert feces and other intestinal contents away from the inflamed mucosa, thereby, decreasing the mechanical, physical and chemical activities of the intestine and putting the bowel at rest (Brown 1913; Goligher 1980; Attisha 1982). Complete bowel rest which follows the cessation of oral and solid food intake, either with parenteral nutrition (Fischer *et al.* 1973; Truelove and Jewell 1974; Fazio *et al.* 1976; Greenberg *et al.* 1976; Babb 1978; Driscoll and Rosenberg 1978; Elson *et al.* 1980; Muller *et al.* 1983) or chemically defined liquid elemental diets (Giorgini *et al.* 1973; Voitk *et al.* 1973), has often been useful in alleviating the symptoms of patients with inflammatory bowel disease. Similarly, fecal diversion which minimizes surface trauma and irritation of the inflamed colon, is expected to allow the inflammatory process to subside and promote healing and regeneration of the damaged mucosa, after which bowel continuity might be restored (Zelas and Jagelman 1980; Attisha 1982). However, the effectiveness of bypass surgery in the management of inflammatory bowel disease is a subject of controversy. Although in some cases (Brown 1913; Garlock and Crohn 1945; Garlock *et al.* 1951; Heaton *et al.* 1964; Truelove *et al.* 1965; Kivel *et al.* 1967; Oberhelman *et al.* 1968; Lee 1975, 1976; Harper *et al.* 1983; Lee and Harper 1983), anastomotic enteroenterostomy, enterocolostomy, diverting ileostomy and diverting colostomy have led to sustained clinical remissions, the results reported by many surgeons (Jones *et al.* 1966; Brill *et al.* 1969; McIlrath 1971; Alexander-Williams *et al.* 1972; Young *et al.* 1975; Oberhelman 1976; Homan and Dineen 1978; Zelas and Jagelman 1980) have been unsatisfactory and resection of the diseased bowel was ultimately carried out.

In one series (Jones *et al.* 1966), 19 of 21 patients showed no signs of improvement with the disease persisting in the excluded segment. More recently, Homan and Dineen (1978) reported that in their series, high recurrence rates and persistence of disease in the bypassed loop necessitated a large number of reoperations. Seldom in most of these studies was restoration of bowel continuity achieved (Jones *et al.* 1966; ; McIlrath 1971; Oberhelman 1976).

Others, by contrast, have reported relatively high rates of bowel closure without segmental resection, for patients with Crohn's colitis (Lee 1975, 1976; Harper *et al.* 1983; Lee and Harper 1983). For example, Lee and Harper (1983) stated that in their series of patients with split ileostomies, 30% had intestinal continuity re-established with total bowel conservation. In addition, of 33 patients surgically treated in an earlier study (Lee 1976), 15 had their bowels reconnected, the majority remaining well for over 12 years.

The influence of reduced intraluminal contents on normal intestinal mucosal integrity and epithelial cell proliferation has been documented in a number of animal studies on the colon (Tilson *et al.* 1976; Janne *et al.* 1977; Hagemann and Stragand 1977; Rijke *et al.* 1979a; Stragand and Hagemann 1977; Ryan *et al.* 1979; Morin *et al.* 1980; Storme and Willems 1981; Rainey *et al.* 1983; Delvaux *et al.* 1983, 1984; Majumdar 1984). Interruption of large bowel continuity, whether by proximal colostomy (Tilson *et al.* 1976; Terpstra *et al.* 1981; Williamson 1982; Delvaux *et al.* 1983, 1984), Thiry-Vella fistulae (TVF) (Rijke *et al.* 1979a; Williamson 1978a, 1982; Rainey *et al.* 1983; Menge and Robinson 1983) or self-emptying blind loop (Pon *et al.* 1982) has often resulted in marked hypoplasia and decreased epithelial cell proliferation of the bypassed segment. Similar atrophic changes are provoked by starvation (Hagemann and Stragand 1977; Stragand and Hagemann 1977; Menge and Robinson 1983; Majumdar 1984), parenteral nutrition (Ryan *et al*. 1979; Morin *et al*. 1980) and chemically defined diets (Janne *et al*. 1977; Ryan *et al*. 1979; Morin *et al*. 1980; Storme and Willems 1981) which are known to reduce fecal bulk in the colonic lumen. Conversely, these adaptive changes are immediately reversed following restoration of bowel continuity (Tilson *et al*. 1976; Terpstra *et al*. 1981; Williamson 1982) and refeeding (Hagemann and Stragand and Hagemann 1977; Williamson 1982; Majumdar 1984).

The colonic hypoplasia and hypoproliferation which result from decreased intraluminal load have been attributed to the loss of mechanical stimulus provided by fecal bulk (Stragand and Hagemann 1977; Ryan *et al*. 1979; Williamson 1982; Menge and Robinson 1983; Delvaux *et al*. 1983, 1984). Mechanical distention is thought to play a very important role in maintaining colonic architecture and epithelial cell proliferation. Other factors, systemic for example, are also involved as is shown by the compensatory changes which occur in the bypassed small bowel after colonic bypass without any loss of tissue (Tilson and Wright 1970), in Thiry-Vella fistulae following resection in rats (Hanson *et al*. 1977) and in bypassed colonic segments after refeeding of starved rats (Delvaux 1984).

In contrast to the atrophic and hypoplastic changes provoked by reduced intraluminal load, increased epithelial cell proliferation and hyperplasia have been described in the colonic mucosa during certain phases of inflammatory bowel disease (Bleiberg *et al*. 1970; Eastwood and Trier 1973; Eastwood 1977; Biasco *et al*. 1979; Lipkin 1981; Serafini *et al*. 1981, Butt *et al*. 1983) and in experimental colitis (Watt and Marcus 1970; Pon *et al*. 1982). The adaptive response to colonic bypass, therefore, might be expected to reverse the hyperplastic and kinetic changes induced by the inflammatory process and so induce healing; not unlike the role played by fecal diversion in the impairment of colonic carcinogenesis in rats (Rainey *et al*. 1983). However, reports on the effectiveness of bypass surgery in the healing and treatment of inflammatory bowel disease have been contradictory (Lee and Harper 1983) and these have been based primarily on a limited number of uncontrolled, clinical studies on small numbers of patients.

No major animal experimentation has been done in an attempt to confirm or supplement the results of these studies, especially histologically and kinetically. The report of Pon *et al*. (1982) appears to be the only account on how experimental diffuse inflamed colonic mucosa of animals is affected by exclusion bypass. This study was very brief, incomplete and its preliminary results require confirmation and extension. According to Young *et al*. (1975), the use of this type of bypass in the treatment of

IBD deserves further evaluation and as Goligher (1980) points out, there are no convincing data which establish the inferiority (or superiority) of bypass over other types of surgery used to treat IBD, resection for example.

The purpose of the present work is, therefore, to assess the effect of colocolic bypass on the experimentally inflamed colon of the male Sprague-Dawley rat, according to the following criteria:

(1) by investigating at the light microscopic level the mucosal morphology of the entire colon, both quantitatively and morphometrically

(2) by studying the total colonic epithelial cell proliferative kinetics with autoradiographic methods and mitotic counts

(3) by describing the pattern of mucus production in each colonic segment, in terms of the specific types of epithelial mucins and the number of mucin-secreting cells, with the use of various histochemical methods.

III. MATERIALS AND METHODS

A. Animal Care

One hundred and sixty-eight noninbred male Sprague-Dawley rats (Biosciences Animal Facilities, Uiversity of Alberta) with an average body weight of 250 g at the start of the experiment were utilized in this study. The animals were housed in groups of six (6) in wire-top cages in the Medical Science Building (MSB) Animal Unit (except during experimental procedures) under conditions of controlled lighting, temperature and humidity. Animals were provided *ad libitum* with a standard pellet diet and water except during the 48 h prior to experimentation, during which time they were given full access to a 5% sucrose solution.

B. Experimental Design

Rats were randomly assigned to one of three experimental groups of 6 to 9 animals each:

(1) An experimental group of animals with an inflamed descending colon prior to bypass

(2) One control group of animals with an inflamed mucosa but no bypass

(3) A second control group that was not inflamed but had bypass surgery

Additionally, 12 untreated rats served as normal non-operated controls.

C. Inflammation Treatment

Experimental diffuse, nonspecific mucosal inflammation of at least the descending colon was induced using the acetic acid technique of MacPherson and Pfeiffer (1978). Rats were slowly administered 0.5 ml of of 10% acetic acid intrarectally (i.r.) under light ether anaesthesia following a starvation period of 48 h. For i.r. instillation, a polyethylene tubing applicator was inserted into the rectum as far up as the splenic flexure. After the solution was instilled it was left *in situ* for 10 seconds, following which the remaining inflammatory agent was withdrawn and the colon flushed three times with 0.5 ml of normal saline (0.9% NaCl).

D. Bypass Procedure

Colonic bypass operations (Figure III.1) were performed under ether anaesthesia 24 h following the acetic acid treatment. The surgical procedure employed to exclude the greater part of the colon from the fecal stream was an end-to-side anastomosis using a single layer of interrupted 5-0 silk sutures, placed about 2 mm apart.

At laparotomy, the ascending colon was transected approximately 2.0 to 2.5 cm distal to its origin from the cecum. Its proximal stump was then sutured to the distal end of the descending colon, as far down as the pelvic girdle would allow; the cecum being left in the fecal stream. At the same time, the remaining cut end was sutured off to

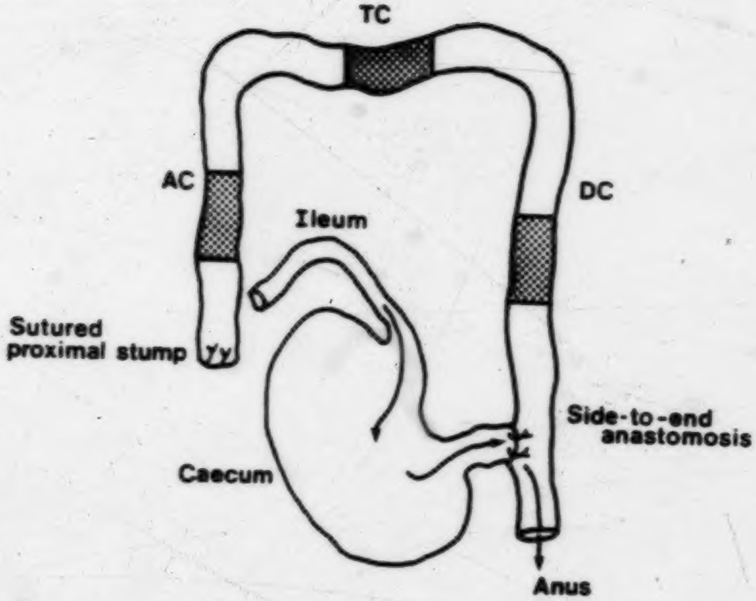

TC
AC
DC
Ileum
Sutured proximal stump
Side-to-end anastomosis
Caecum
Anus

Figure III.1 Surgical Procedure (Shaded areas represent the sites from which specimens were resected).

create a blind loop. The abdominal incision was closed with one layer of continuous 'thread' suture.

E. Tissue Handling and Staining

Animals were sacrificed at 1, 2, 3, 4, 6, 8, 10, and 12 weeks following establishment of the bypass. At each sampling interval, at least six animals from each experimental group were brought to the laboratory where 3 rats from each group were weighed before being injected with tritiated thymidine (to be described below). All animals were sacrificed between 0900 h and 1200 h to minimize the effects of diurnal variation on cell proliferative activity.

Under ether anaesthesia, the abdomen was opened and the splenic and hepatic flexures pinned to delineate the extent of the transverse colonic segment. The entire colon was removed in continuity with the cecum by transecting the small bowel and cutting the distal colon at the pelvic brim. Two adjacent tissue blocks were routinely resected from mid-ascending, transverse and descending colonic regions. One was opened longitudinally, washed thoroughly in normal saline to remove fecal material and pinned to cork blocks, mucosal side up. Tissues were fixed for at least 24 h in 10% neutral buffered formalin. Subsequently, the flattened 'cork block' tissue was divided into two pieces; one being used for paraffin embedding, to be described later, while the other was further divided into two smaller pieces to be processed through a graded series of ethanol and properly oriented (one to be sectioned frontally, the other in the longitudinal axis of the intestine) prior to glycol methacrylate (JB-4 embedding medium; Polysciences, Inc. Warrington, Pa.) embedding. The adjacent intact tubal specimen was similarly handled.

Two micrometer (um) thick sections were cut on a Sorvall Porter-Blum JB-4A microtome. They were floated onto precleaned microscope slides to which they were heat-fixed on a slide warmer set at approximately 40°C. The slides were stained for 2 minutes in Lee's (Bennett *et. al.* 1976) methylene blue-basic fucshin (MBBF), its working solution phosphate-buffered to pH 7.2. They were then rinsed in distilled water, dipped in 50% ethanol (ETOH) for the removal of excess stain and air dried before being mounted in plastic mounting media (Polysciences Inc., Warrington, Pa.) or Permount (Fisher Scientific).

F. Autoradiographic Methods

Labelling of Animals

At each sacrifice interval, three animals from each experimental group were weighed and then injected intraperitoneally with tritiated thymidine (3HTdR; ICN Pharmaceuticals, specific activity 60-90Ci/mmole) in about 0.1 ml of sterile water, at a dose level of 1 uCi/g body weight, between 0900 h and 1100 h, to minimize the effect of circadian rhythm (Chang 1971; Hamilton 1979; Potten *et al.*

1977). One hour later, under ether anaesthesia, tissue samples were excised, processed and embedded in methacrylate as described earlier.

Autoradiography

Autoradiographs were prepared from two micrometer thick methacrylate sections. Slides placed in plastic slide grips (Lipshaw, Detroit, Mich.) were dipped in Kodak NTB2 nuclear track emulsion for approximately 3 seconds. They were then dried for 60 min in an incubator controlled at 28°C and 80% humidity. The slides were subsequently exposed for 4 weeks at 4°C in sealed, light-tight, black bakelite boxes (Seargent Welch, Toronto, Ontario) containing sacs of indicating drierite, after which they were developed in Kodak Dektol 1:1 for 2 min at room temperature (25°C), without agitation. They were further rinsed in distilled water for 10 sec, fixed in Kodak Fixer (Eastman Kodak Co., Rochester, N.Y.) for 5 min, rinsed for 5 min in slowing running water and dried in a humidity-controlled incubator. The slides were then stained for 2 min in MBBF, dried, mounted and coverslipped.

G. Histological Analysis

Several parameters were assessed on the light microscope via ocular micrometry and cell counting, from coded slides of two micrometer thick methacrylate-embedded sections. For the study of most morphological parameters, 20 (twenty) or occasionally, 15 (fifteen) separate perfect axially sectioned crypts of Lieberkuhn, open from top to bottom, as well as 20 cross-sectioned glands were analyzed from each colonic segment of each animal.

Quantitative Morphological Parameters

For quantitative analysis, crypts were scored for:

(1) **The number of epithelial cells per crypt column**; this parameter was used as one estimation of crypt length and was determined by enumerating, from the mid-point of the crypt base to the crypt mouth (crypt-surface junction), the number of cells (nuclei) found in one half of longitudinally cut glands (the crypt column) as described by Chang and Leblond (1971) and Watson *et al.* (1982).

(2) **The column count**, as expressed by the number of epithelial cells (nuclei) per circumference of cross-sectioned crypts seen in frontal sections of the colon (Rijke *et al.* 1979a).

(3) The total number of epithelial cell per crypt (**the crypt cell population**), estimated from the product of crypt column and column count values (Sunter *et al.* 1979).

As well, from frontal sections of colonic segments was scored:

(4) **The number of transversely sectioned crypts per unit area** seen in three random areas. This was determined

by counting all complete glands seen in the field indicated by the 'circle' of the ocular micrometer as well as those cut by the left half of the field (clockwise 6 to 12 h), but not those cut off by the right half of the field (clockwise 12 to 6 h); similar to the method described by Rubio and Kock (1981). Glands in the ascending colon were counted with the 40X objective, while those of the transverse and descending segments were counted with the 10X objective.

Morphometric Parameters

For morphometric analysis, several aspects of mucosal morphology were determined by the use of ocular micrometry. Measurements were made in micrometers (um), using a 10 mm eyepiece micrometer and a 10X or 40X objective. The following parameters were obtained from the axially sectioned crypts (See Figure III.2):

(1) the **minor crypt width** (diameter); the distance from cell surface to cell surface across the lumen of the crypt at the:

  (a) apex (D, on luminal side of colon) of the crypt
  (b) medial portion (D_1, with respect to crypt length)
  (c) base (D_2, serosal side)
  (after Gutschmidt *et al.* 1983)

(2) the **major crypt width** (diameter), the distance from

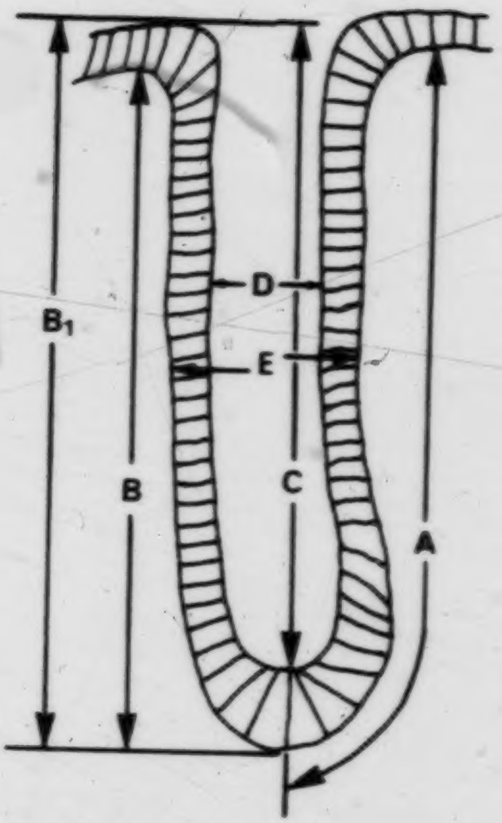


Figure III.2 Longitudinal Crypt Parameters

A = Crypt column (cells).
B = Distance from basement membrane of crypt base cells to that of the surface epithelium.
B_1 = Distance from crypt base to tip of surface epithelium.
C = Distance from apex of crypt base cells to tip of surface epithelium.
D = Minor crypt width.
E = Major crypt width.

basement membrane to basement membrane across the crypt at the above corresponding:

(a) apex (E)

(b) medial portion (E_1)

(c) base (E_2)

(3) The distance from the basement membrane of the cells at the base of the crypt to the basement membrane of the cells of the surface epithelium (B)

(4) The distance from the apex of basal crypt cells to the tip of the surface epithelium (C)

(5) The distance between the crypt base and and the tip of the surface epithelium (B_1)

and from the 20 cross-sectioned crypts was recorded:

(6) The **minor diameter** (X), that is, the luminal diameter or the distance from cell surface to cell surface.

(7) The corresponding **major diameter** (Y), the distance from basement membrane to basement membrane

Mucosal Proliferative Activity

Mucosal proliferation was assessed from the longitudinally cut crypts according to the following criteria:

(1) by counting the number of mitotic figures and measuring the position of each one

(2) by recording the height of the **proliferative zone** expressed as:

(a) the **leading edge** - the distance measured from the base of the crypt to the tip of the highest labelled nucleus (considered labelled if it was overlaid by at least three grains).

(b) the distance of the highest mitotic figure from the base of the crypt

(3) by estimating the **mitotic index**, expressed as the percentage of mitotic figures per total number of crypt column cells (Heitman *et al*. 1983)

Statistical Analysis

Statistical analysis was carried out using analysis of variance, Scheffe's multicomparison tests and Pearson correlation coefficients. The SPSS-X statistical package was used to analyze the data.

H. Histochemical Methods For Mucin Identification

Tissue specimens used for the demonstration of colonic mucous cells and their epithelial mucins (glycoproteins) were routinely fixed in 10% neutral buffered formalin, dehydrated in a graded series of ethanol, cleared in chloroform or xylene (See Appendix 1) and embedded in paraffin (Paraplast, Fisher Scientific). Six micrometer (6um) serial step sections from these blocks were stained using a number of histochemical procedures to demonstrate, by simultaneous visualisation in contrasting colours, the specific types of epithelial mucins found in different regions of the crypt of Lieberkuhn and in each anatomical segment of the large bowel, as well as the number of mucin-containing cells per longitudinal crypt of each colonic segment. The histochemical stains used were as follows:

(1) **The Alcian Blue pH 2.5 - Periodic Acid Schiff (AB pH 2.5/PAS)** (Mowry and Morard 1957; Mowry 1963)

In this sequence, mucous cells containing neutral mucins stained red, those with acid mucins stained blue while those containing a mixture of the two glycoproteins stained purple. For details of this procedure, see Appendix 3.

(2) **The Saponification - Alcian Blue pH 1.0 - Periodic acid - Phenylhydrazine - Schiff (KOH/AB pH 1.0/PAPS)** procedure

This technique was performed as described by Reid *et al.* (1985). It was used for the differential staining of the two types of acid mucins; mucous cells containing O-sulphate ester (sulphomucins) stained blue; those with sialic acids (sialomucins), with or without side chain substituents, stained red; whereas those containing a mixture of the two acid mucins stained various shades of purple, indicative of the relative amounts of these two glycoproteins.

(3) **The Periodic Acid - Phenylhydrazine - Thionin Schiff - Saponification - Borohydride Reduction - Periodic**

Acid - Schiff (PAPT/KOH/Bh/PAS) procedure of Reid *et al.* (1984c)

This procedure was employed to distinguish between sialic acids that were unsubstituted and those that were substituted at the side chain. With this sequence, mucous cells comprised exclusively of unsubstituted sialic acids were blue in colour. By contrast, those with O-acyl substituents only stained red. However, those cells with various proportions of substituted and unsubstituted sialomucins stained in various shades of purple, ranging from blue purple to red purple. For example, cells with a one-to-one ratio of these were visualized as purple whereas those containing a predominance of substituted sialic acids (traces of unsubstituted SA) stained red purple.

IV. RESULTS

A. Crypt Height

Morphometric

The morphometric data for crypt height are presented in Table IV.1 and Figure IV.1. In terms of measurement (micrometers), crypts in the ascending and descending colon are similar in length (238.9 vs 249), but are shorter than those of the transverse colon (275.3).

As Tables IV.1 and IV.2 show, following surgery in the bypass controls, with the exception of the first time interval, crypt height was not affected significantly in the descending colon while it was significantly reduced in the ascending and to a lesser extent in the transverse colon. Glandular atrophy rapidly developed in the excluded ascending colon (Figure IV.2). At week 1, crypts were cut to about half their normal size (121.3 for BPCN vs 238.9 for controls). Furthermore, with time, morphometric values remained significantly below non-operated controls.

The transverse colon exhibited a similar atrophic pattern (Figure IV.3). The difference from control ($P < 0.05$), however, was not as pronounced. Although shorter crypts were evident as early as 1 week postoperatively and persisted throughout the experiment, reduced crypt depth reached only 75% to 89% of control values compared with the 51% to 71% observed in the ascending colon.

Table IV.1. Crypt Height in Micrometers

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	238.9±6.3[1] (12)[2]	228.7±2.2 (6)	121.3±5.6 (6)[b]	202.6±30.3 (6)	282.2±9.9 (6)	168.5±13.6 (6)[a]	151.2±5.4 (6)	258.1±10.1 (7)	144.7±6.8 (6)[a]	174.7±13.1 (6)	262.4±9.2 (7)	128.6±2.7 (5)[a]	150.2±8.2 (6)
Transverse	275.3±6.2 (12)	331.1±32.7 (6)	220.9±9.7 (6)[b]	314.2±30.4 (6)	348.5±18.2 (6)	236.7±7.5 (6)[a]	253.4±9.0	353.6±19.5 (8)	244.4±5.1 (6)[a]	287.2±8.2 (6)	333.5±10.8 (8)	238.3±7.8 (6)[a]	264.7±12.6 (6)
Descending	249.2±3.3 (12)	478.3±16.5 (6)	225.2±10.8 (6)[b]	401.3±35.5 (6)	471.6±25.1 (6)	258.1±4.5 (7)[d]	347.8±23.5	427.9±12.7 (7)	263.6±10.3 (6)[a]	300.1±6.4 (6)	364.2±24.9 (6)	249.7±7.0 (6)[a]	288.8±18.0 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	216.5±9.0 (6)	120.1±4.6 (6)[e]	199.9±41.8 (5)	243.3±2.9 (6)	142.6±9.4 (6)[a]	157.7±8.6 (6)	257.7±4.4 (7)	126.4±4.9 (6)[a]	138.1±4.7	253.7±5.1 (6)	142.6±7.2 (6)[a]	165.6±10.1 (6)
	297.1±22.9 (6)	208.8±8.5 (6)[e]	302.6±37.2 (5)	270.4±8.6 (6)	215.8±8.5 (6)[a]	263.1±10.8 (6)	263.1±5.9 (7)	207.4±10.4 (6)[a]	219.8±7.7 (6)	257.8±8.8 (9)	221.9±6.4 (6)[a]	238.3±4.9 (6)
	323.3±22.4 (6)	249.2±6.0 (6)[e]	332.5±42.4 (5)	283.6±11.9 (6)	220.4±5.7 (7)[a]	271.5±17.5 (6)	251.3±5.4 (6)	224.9±6.5 (6)[a]	230.5±8.6	253.1±6.9 (9)	232.7±5.3 (6)[a]	250.6±11.9 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	4.76	<0.001	<0.05

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For [3]Scheffe's Multiple Comparisons:

[a] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[b] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.

[c] No significant differences between the 3 groups.

[d] Significantly different from each other.

[e] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

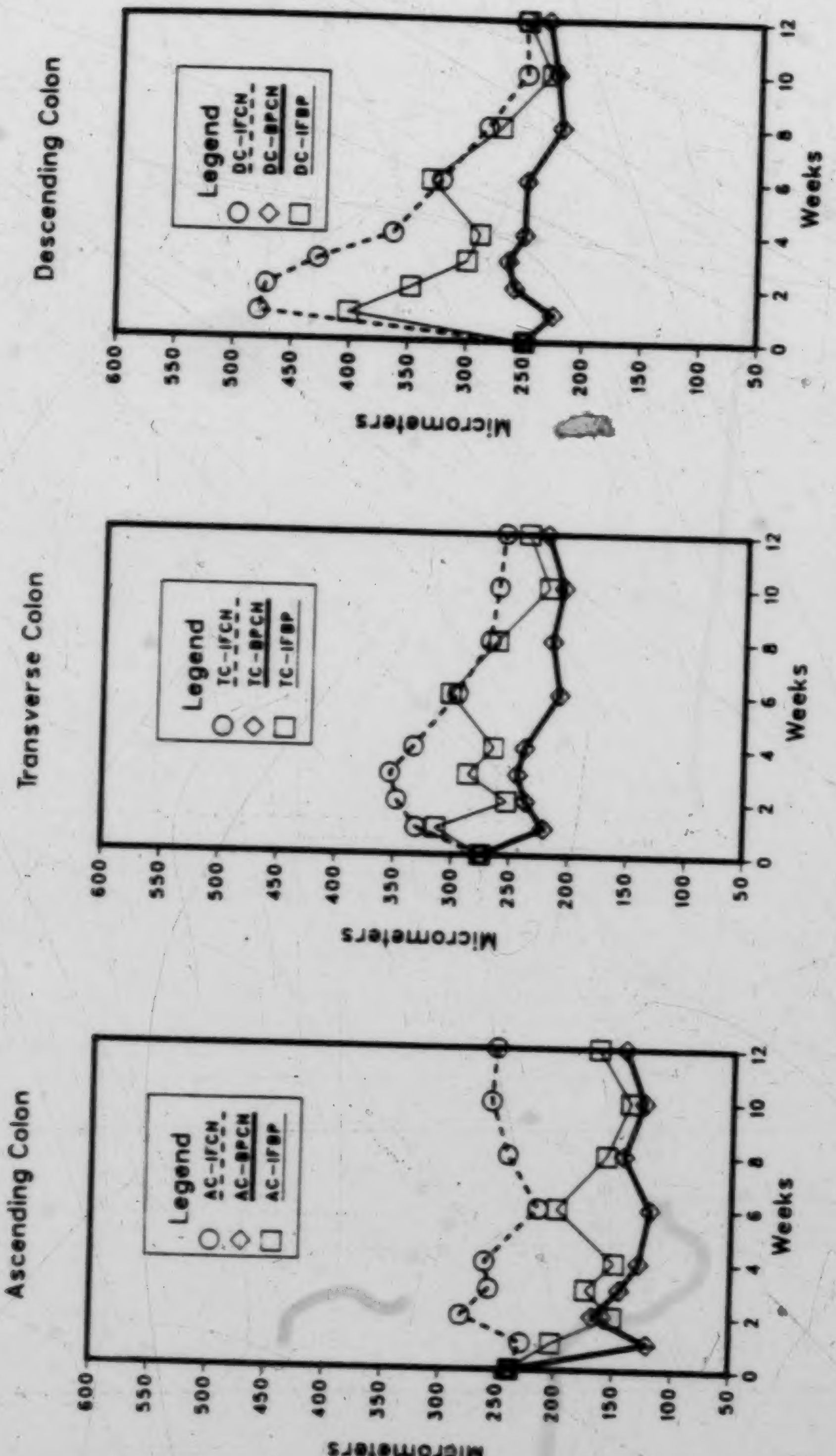


Figure IV.1. Crypt Height in Micrometers (Control: x=0,y)

Table IV.2. Scheffe's Multiple Comparison Test (Group x Segment, $P<0.05$). Variables Showing Significant Differences

Ascending Colon

	Control	BP/CN	IF/BP	IF/CN
BP/CN	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, PZ, F_1, MEANE, D, E, E_1, E_2, Y, J	—	—	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, PZ, F_1, F_1/A, MEANE, D, E, D_1, E_1, E_2, Y, J
IF/BP	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, MEANE, D, E, E_1, E_2, Y, J	—	—	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, MEAND, MEANE, D, E, D_1, E_1, E_2, Y
IF/CN	—	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, PZ, F_1, F_1/A, MEANE, D, E, D_1, E_1, E_2, Y, J	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, MEAND, MEANE, D, E, D_1, E_1, E_2, Y	
Control	—	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, PZ, F_1, MEANE, D, E, E_1, E_2, Y, J	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, MEANE, D, E, E_1, E_2, Y, J	—

Transverse Colon

	Control	BP/CN	IF/BP	IF/CN
BP/CN	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, MEANE, E, Y, J	—	A, B, B_1, C	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, PZ, F_1, MEAND, MEANE, D, E, D_1, E_1, E_1, X, Y, J
IF/BP	Z, AZ, F_1, F_1/A, Y, J	A, B, B_1, C	—	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, F_1, F_1/A, MEAND, MEANE, D, E, D_1, E_1, E_2, Y; J
IF/CN	A, AZ, MEAND, D, D_1	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, PZ, F_1, MEAND, MEANE, D, E, D_1, E_1, E_2, X, Y, J	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, FHMB, F_1, F_1/A, MEAND, MEANE, D, E, D_1, E_1, E_2, Y, J	—
Control	—	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, MEANE, E, Y, J	Z, AZ, F_1, F_1/A, Y, J	A, AZ, MEAND, D, D_1

Table IV.2 (Cont'd)

Descending Colon

	Control	BP/CN	IF/BP	IF/CN
BP/CN	Z	—	A, AZ, B, B_1, C, D	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, PZ, F_1, MEAND, D, X, Y
IF/BP	A, B, B_1, C, D	A, AZ, B, B_1, C, D	—	A, AZ, B_1, C, F_1, D
IF/CN	A, AZ, B, B_1, C, FBMEAN, FBMAX, PZ, F_1, MEAND, D, D_1	A, Z, AZ, B, B_1, C, FBMEAN, FBMAX, PZ, F_1, MEAND, D, X, Y	A, AZ, B_1, C, F_1, D	—
Control	—	Z	A, B, B_1, C, D	A, AZ, B, B_1, C, FBMEAN, FBMAX, PZ, F_1, MEAND, D, D_1

A = Column height in number of cells.
Z = Column count.
AZ = Total crypt cell population (A•Z).
B = Crypt height in micrometers.
B_1 = Distance from crypt base to tip of surface epithelium.
C = Distance from apex of crypt base to tip of surface epithelium.
FBMEAN = Average height of mitotic figures from crypt base.
FBMAX = Maximum height of mitotic figures.
FHMB = Relative size of proliferative zone (100•FBMAX/B).
PZ = Proliferative zone height (leading edge).
F_1 = Number of mitotic figures in longitudinal crypt.
F_1/A = Mitotic index (100•F_1/A).
MEAND = Average minor crypt width (mean of D, D_1, D_2).
MEANE = Average major crypt width (mean of E, E_1, and E_2.
D = Apical minor crypt width.
E = Apical major crypt width.
D_1 = Medial minor crypt width.
E_1 = Medial major crypt width.
E_2 = Basal major crypt width.
X = Minor diameter of cross-sectioned crypts.
Y = Major diameter of cross-sectioned crypts.
J = Number of cross-sectioned crypts per unit area (frontal sections).

Figure IV.2. Comparison of normal and defunctioned ascending colonic mucosae.

A. Normal ascending colonic mucosa which shows bifurcated crypts. MBBF, X330.

B. Specimen taken from the corresponding segment of bypass controls 8 weeks following fecal diversion. Significant mucosal atrophy and hypoplasia are evident. MBBF, X330.

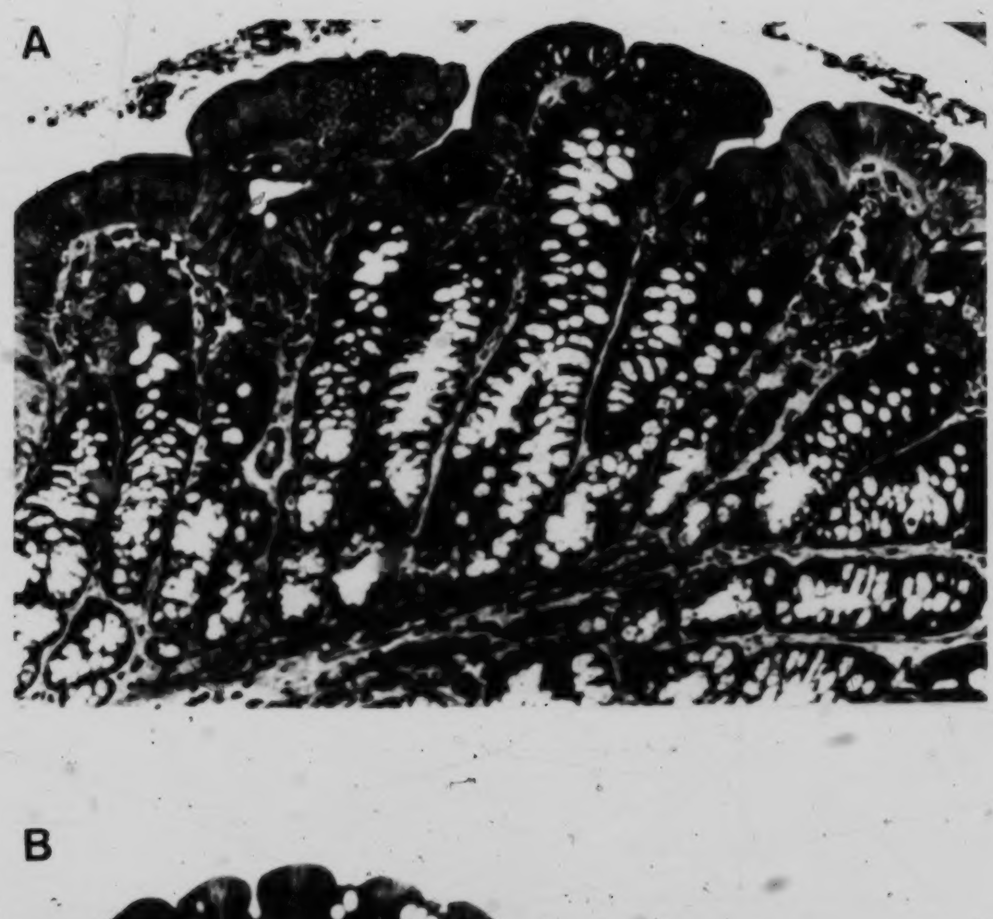
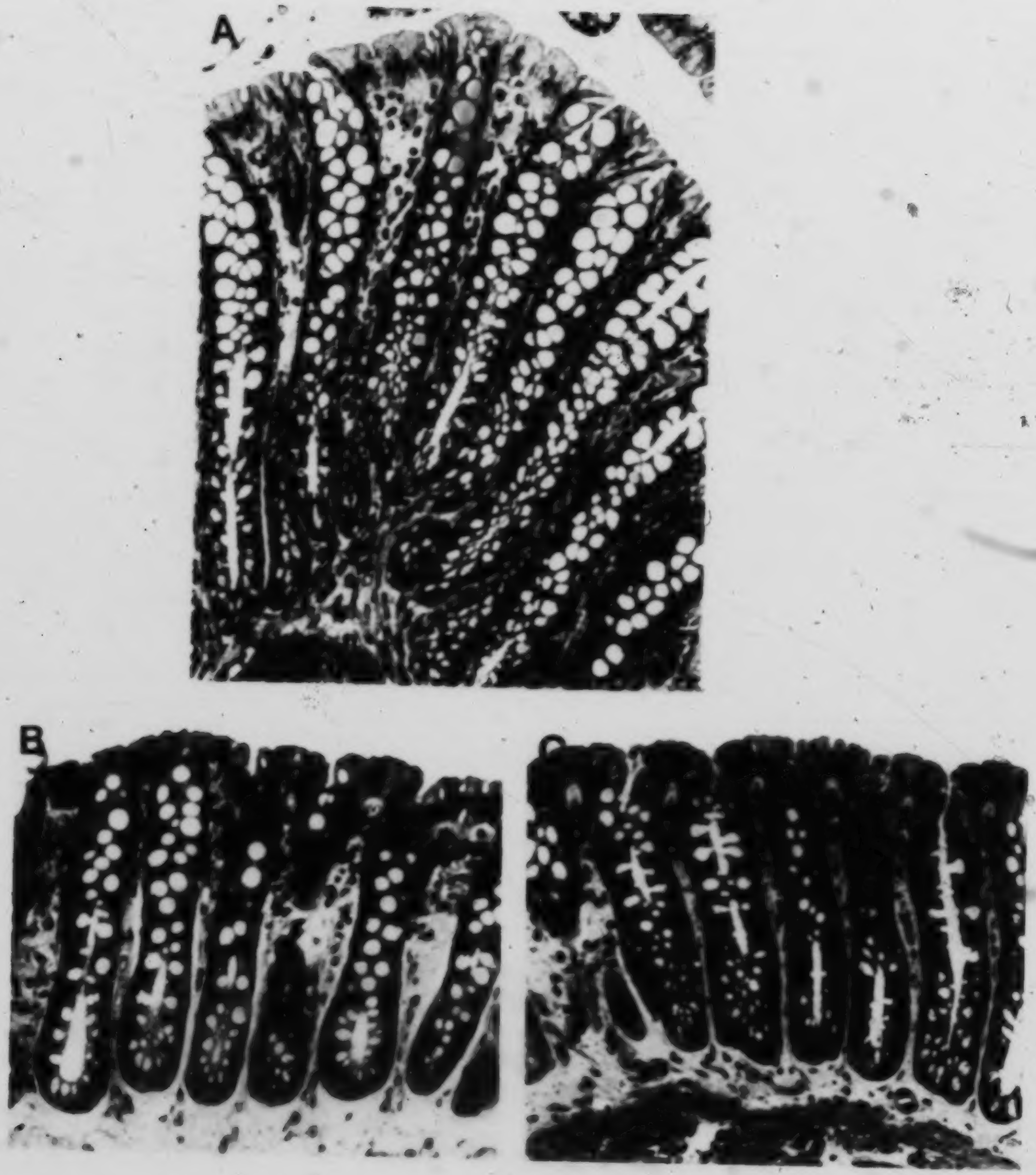

A
B

Figure IV.3. Difference in mucosal height between normal and bypassed transverse colonic segments.

A. Normal transverse colon showing uniform, tubular glands. MBBF, X205.

B. Transverse colon 4 weeks after bypass in the BPCN group. MBBF, X205.

C. Twelve weeks following creation of side-to-end anastomosis in bypass controls. MBBF, X205.


A
B
C

In contrast to the bypass effect, inflammation led to a significant increase in crypt height, peaking at twice the control values within 1 week in the descending colon (Figure IV.4); declining thereafter and reverting to near-control levels at week 8. A significant, though less marked, increase also occurred in the transverse colon (Figure IV.5). However, in this segment peak levels occurred at week 3 and then again steadily fell to near-normal by week 6. Following an apparent delayed reaction, crypt height in the proximal colon also (Figure IV.6), showed a slight, but insignificant, increase at week 2 at which time the peak was reached, before a return to control values at week 4.

As the data from the inflamed-bypass rats show, fecal diversion had a reductive effect on inflamed values (Figures IV.7 and IV.8). In the distal colon, after 1, 2, 3 and 4 weeks of surgery, crypt lengths were respectively, 84%, 74%, 70% and 79% of inflamed controls. The height of the IFBP crypts (288.8 um) at week 4 approximated IFCN values (283.6 um) at week 8. This suggested that despite the irregular values seen at week 6, a near-normal recovery had resulted from bypass about 4 weeks before the inflammatory reaction was terminated in the inflamed controls. Differences between these two groups were insignificant by week 8 (Figure IV.9). A similar trend was exhibited by the transverse colon, except that normal crypt depth was restored within 14 days (Figure IV.10). In the ascending colon bypass negated the transient increase that occurred in the inflamed

Figure IV.4. Distal colonic sections, demonstrating that IFCN crypts peaked at about twice the normal and bypassed controls heights.

A. Week 1 inflamed controls. MBBF, X137.5.

B. Normal controls. MBBF, X137.5.

C. Week 1 bypass controls. MBBF, X137.5.

2

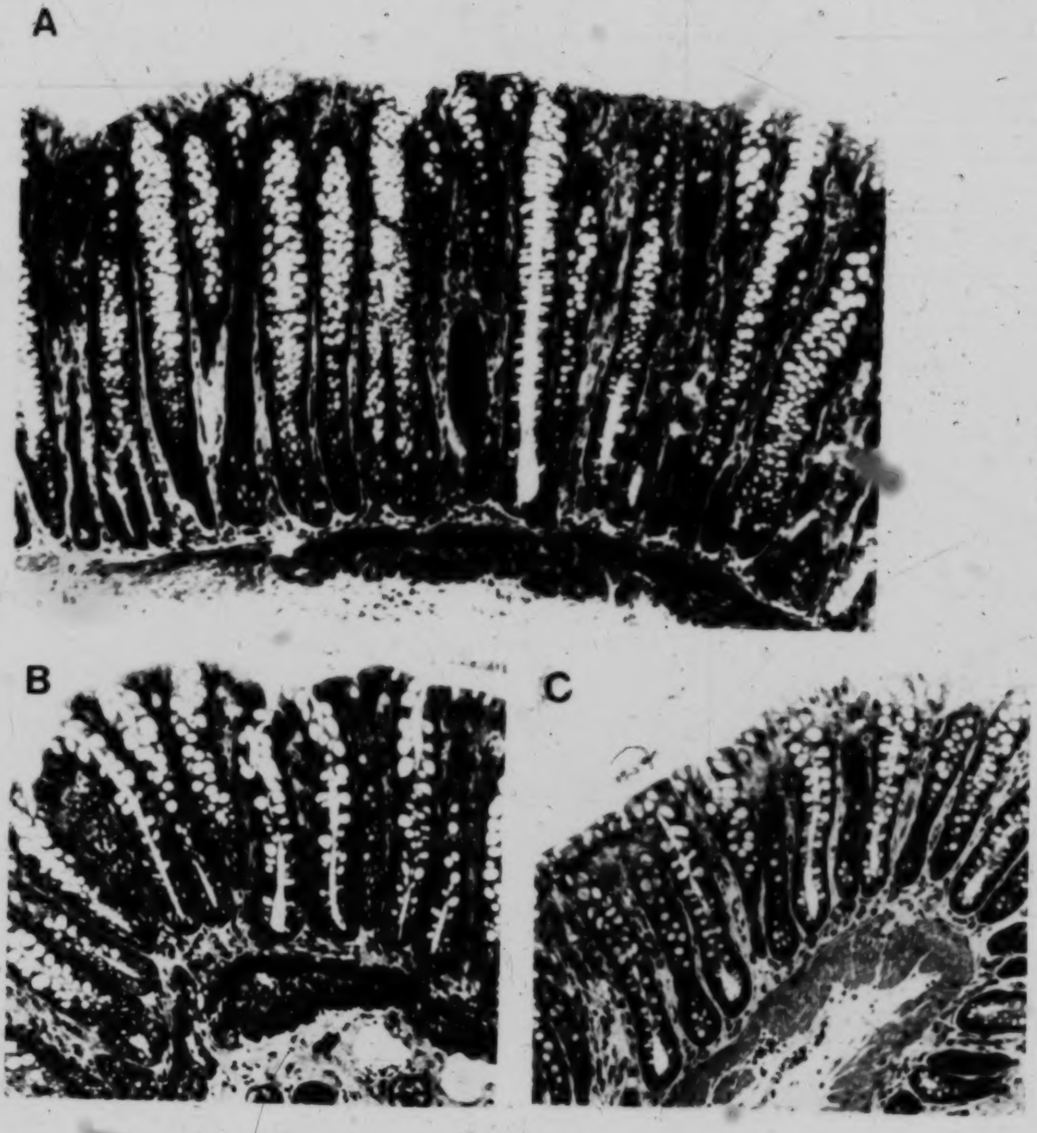


Figure IV.5. Transverse colonic comparison between:

A. Acetic acid-treated mucosa of IFCN animals at 1 week. MBBF, X225.

B. Untreated colonic mucosa. MBBF, X225.

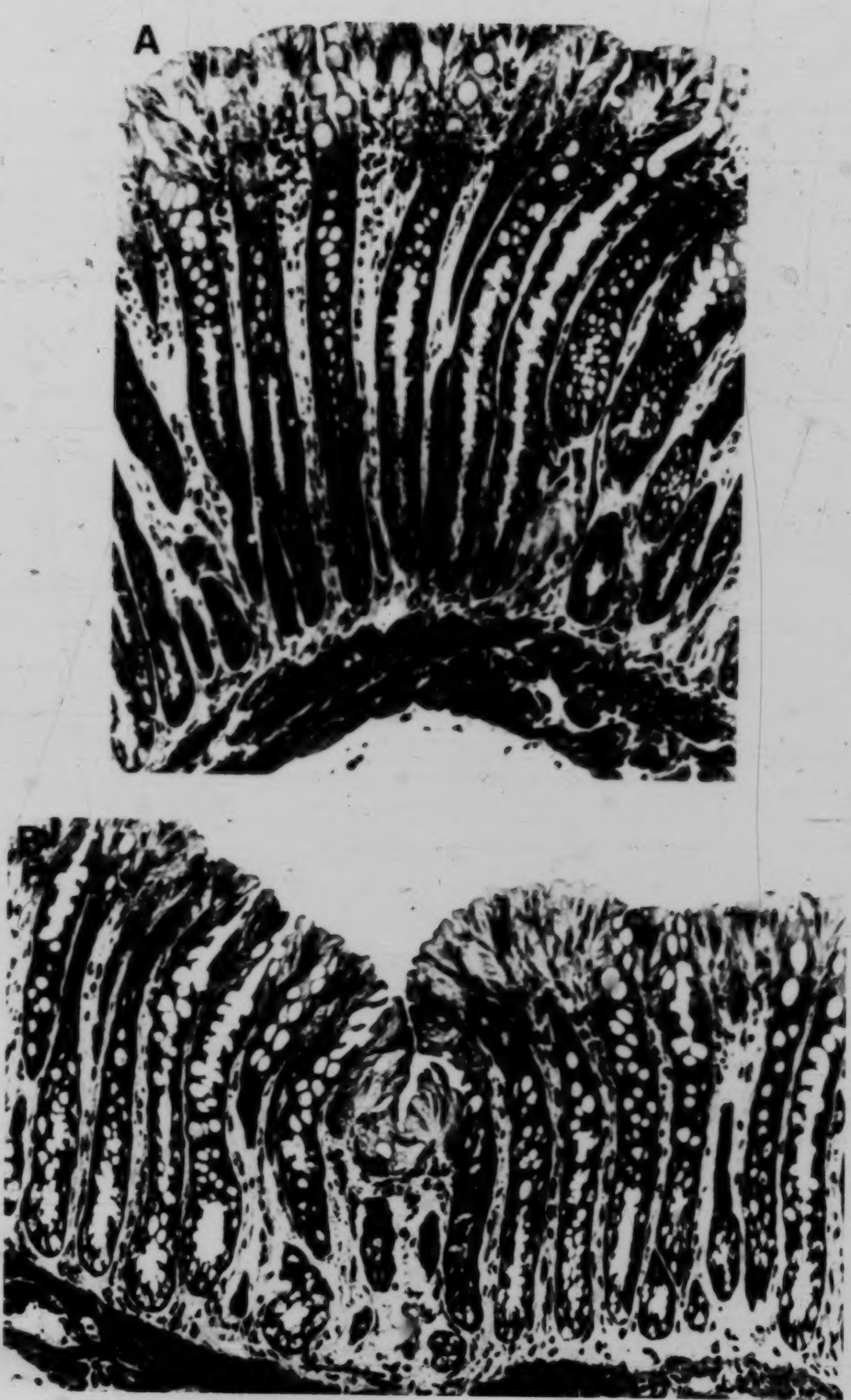

A
B

Figure IV.6. Specimens from ascending colon of normal and inflamed control animals, demonstrating no appreciable difference between the two.

A. Normal proximal colon. MBBF, X225.

B. As A, but at a lower magnification (different animal). MBBF, X137.5.

C. The corresponding normal-appearing segment 1 week after the inflammatory treatment. MBBF, X137.5.

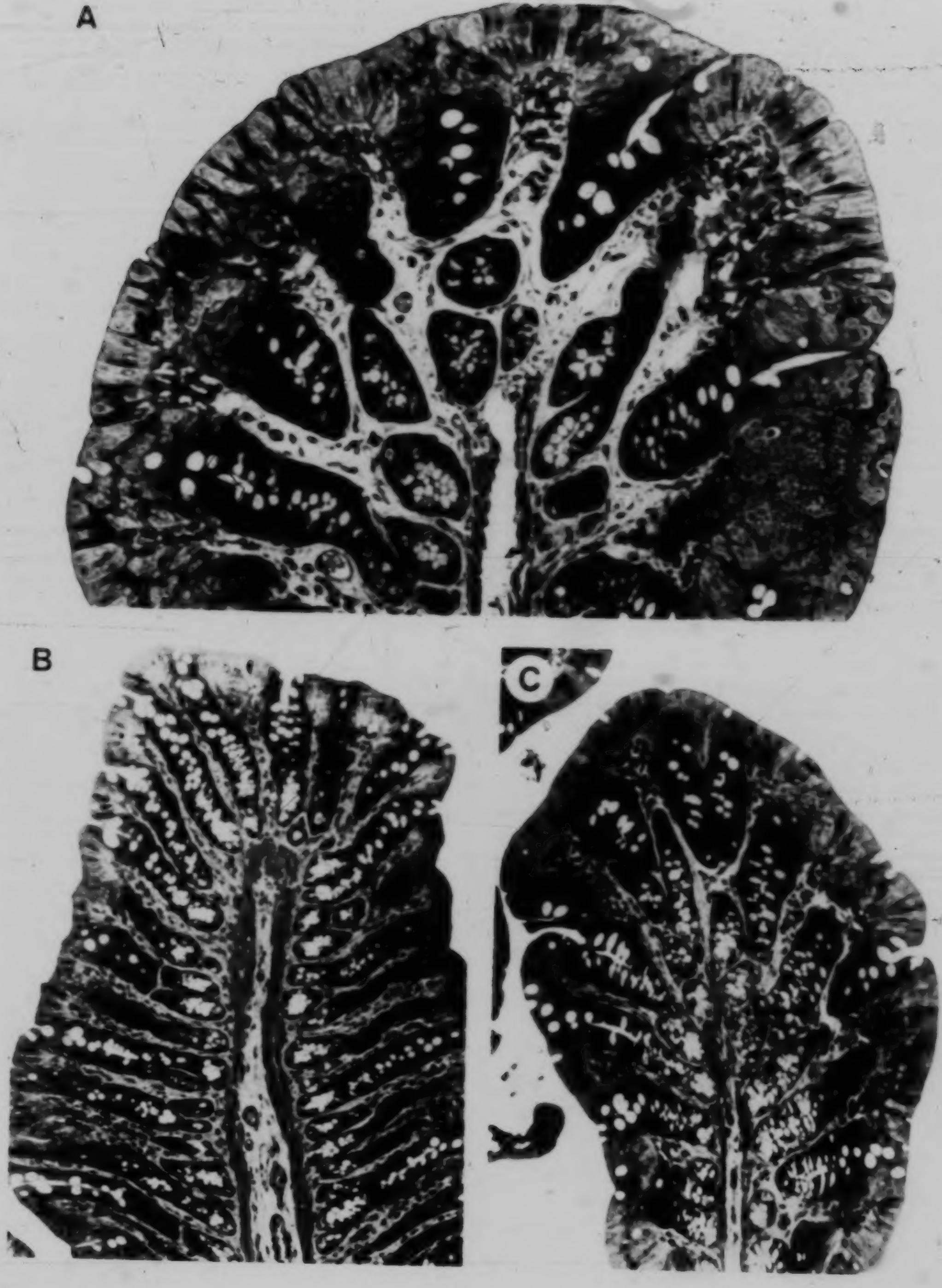

A
B
C

Figure IV.7. Photomicographs of the distal colon at identical magnifications, showing the reductive effect of fecal diversion on experimental colitis observed at week 1.

A. Inflamed colon following bypass. MBBF, X225.

B. Hyperplastic mucosa of inflamed controls. MBBF, X225.

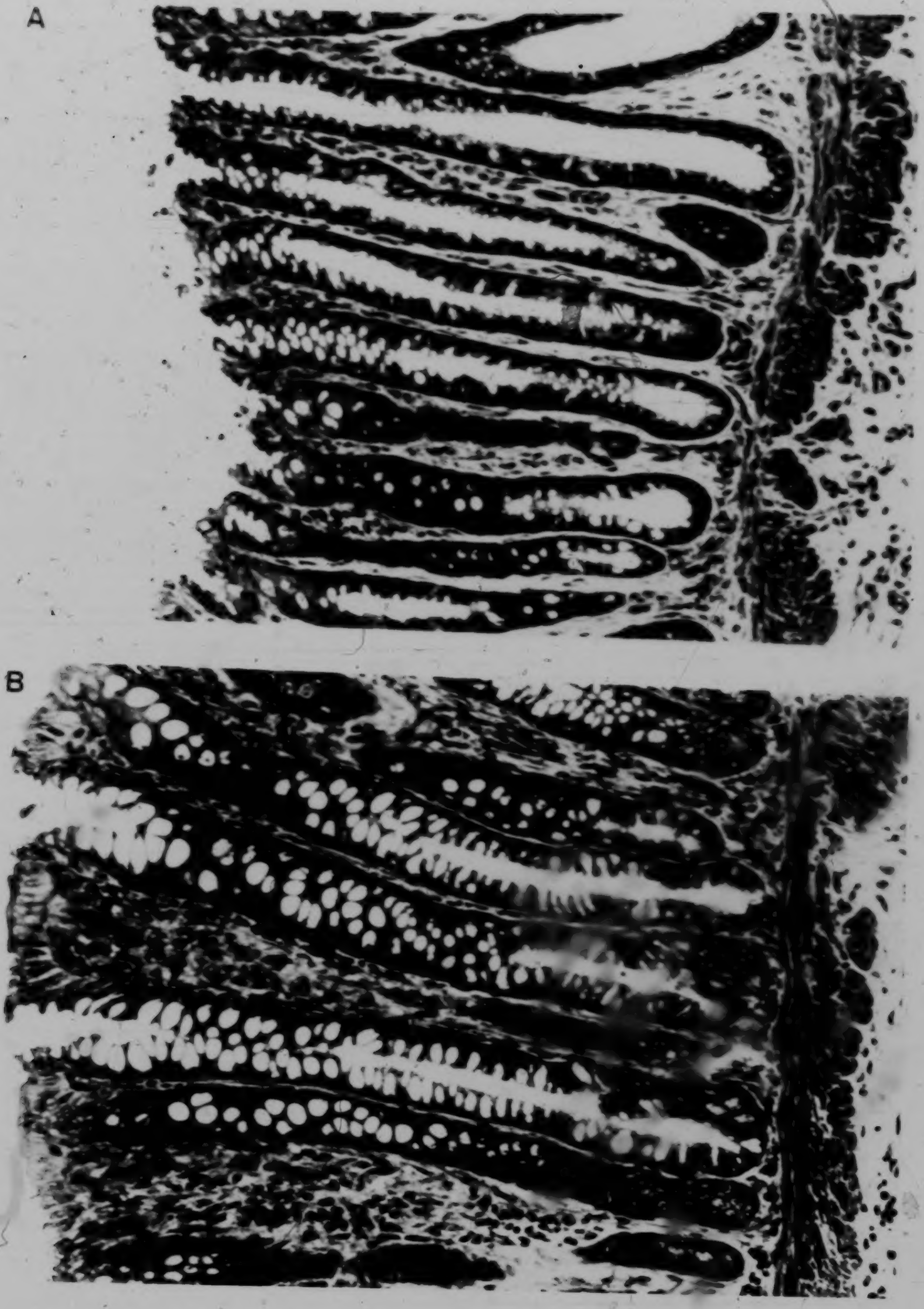
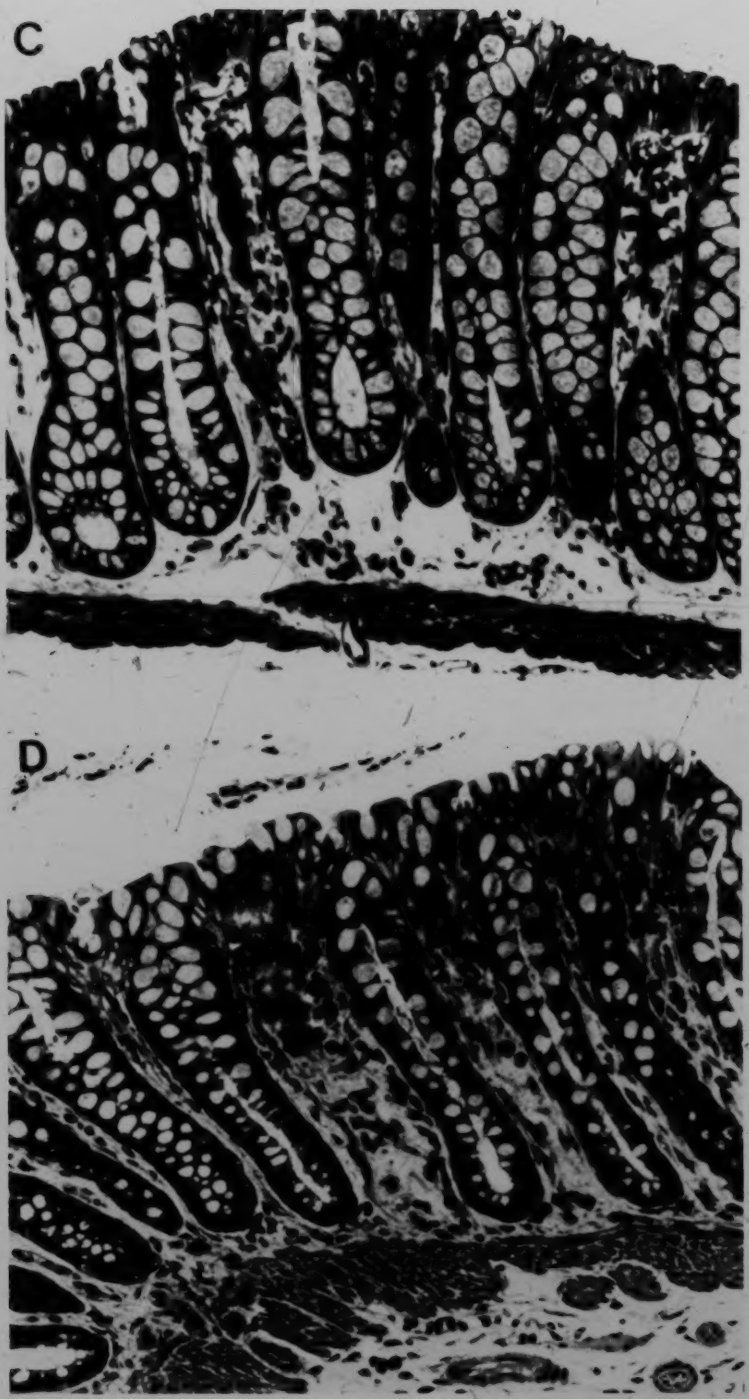

A
B

Figure IV.7. (Cont'd)

C. Descending colonic mucosa of normal controls. MBBF, X225.

D. Corresponding normoplastic segment of bypass-operated animals. MBBF, X225.


C
D

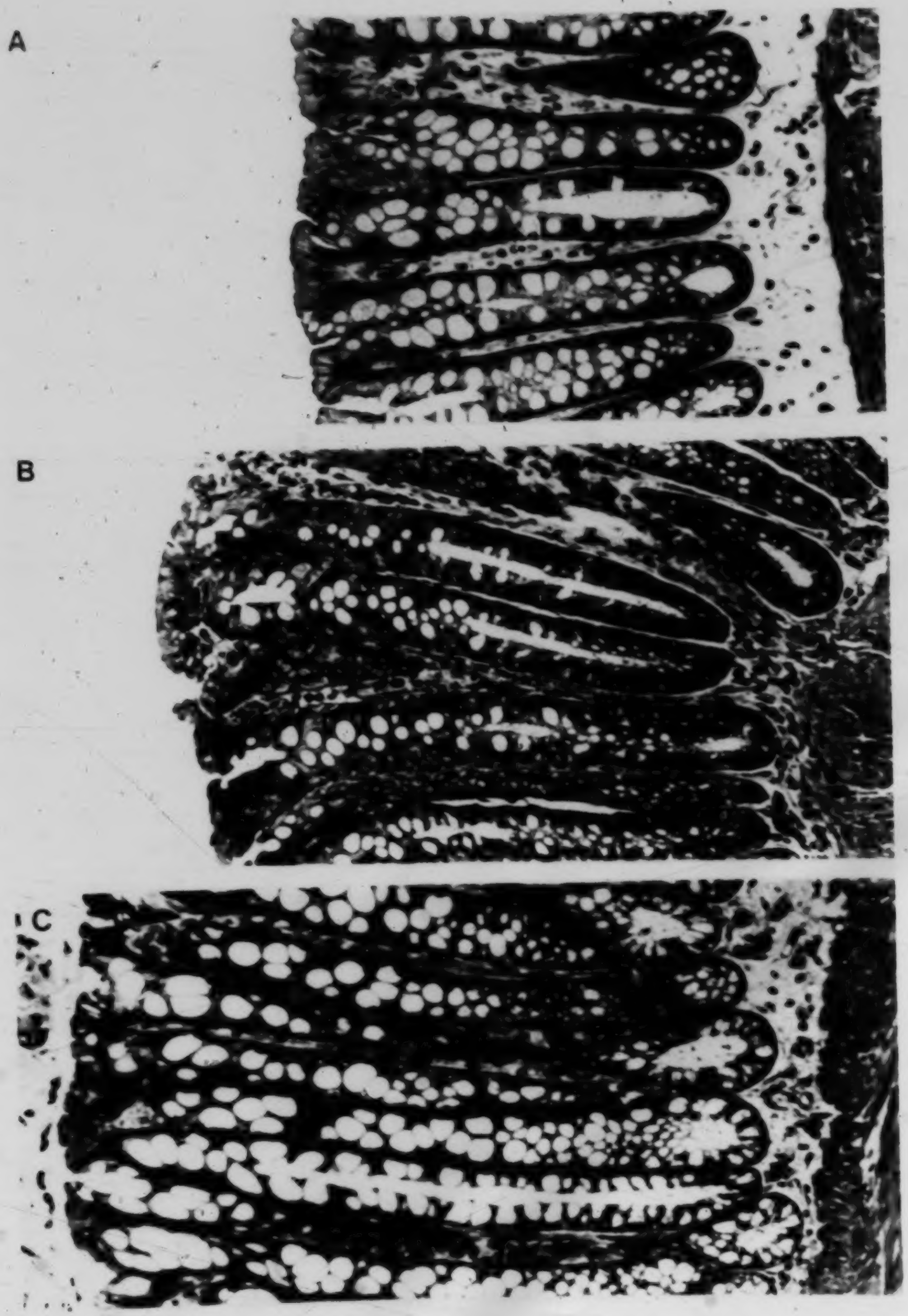

A
B
C

Figure IV.9A. Descending colonic specimen of IFBP rats 2 months following bypass of the inflamed colon showing a near-normal mucosa. MBBF, X225.

B. Specimen taken from the distal colon of an IFCN rat at week 8, demonstrating virtual absence of inflammatory activity. MBBF, X225.

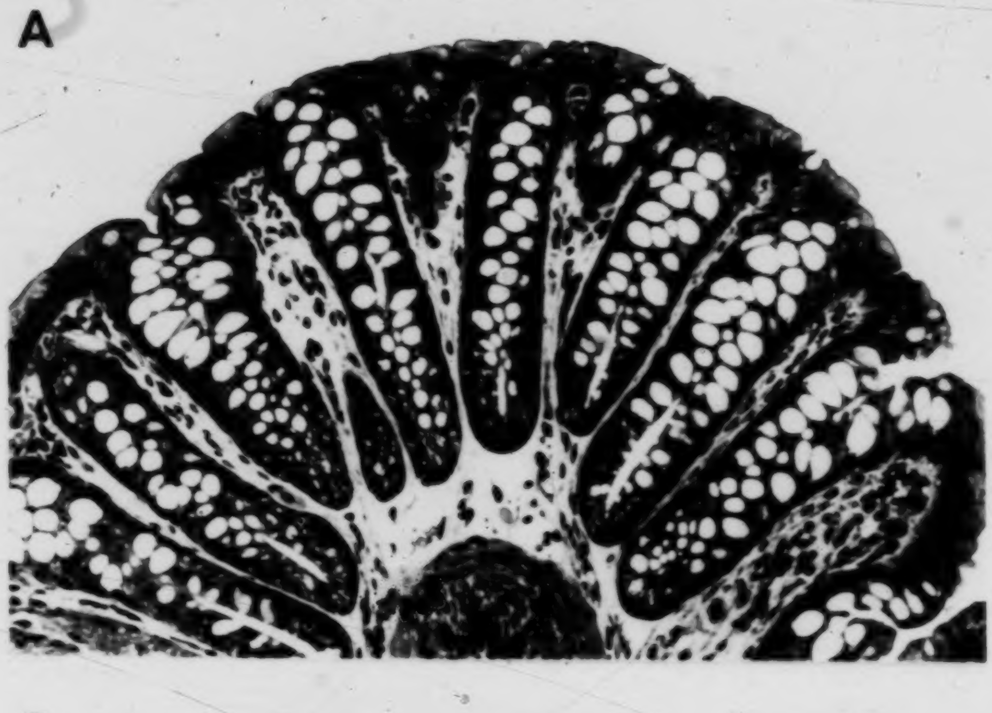
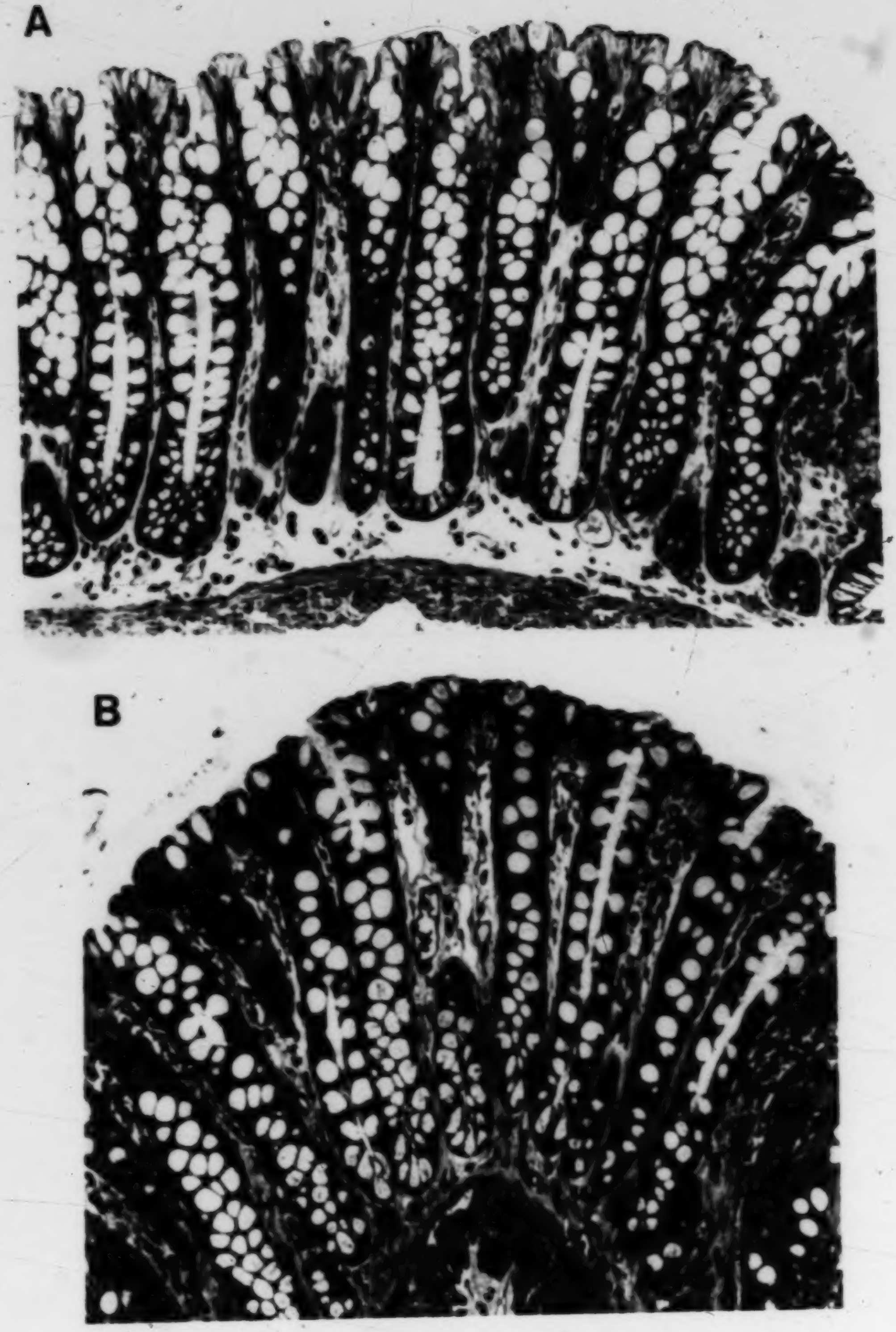

A
B

Figure IV.10. Inflamed transverse colon shows a near-normal return of crypt depth within 14 days of bypass.

A. Normal transverse mucosa. MBBF, X225.

B. Corresponding IFBP segment at week 2. MBBF, X225.


A
B

controls.

Column Height in Number of Cells

Column length in number of cells (Table IV.3, Figure IV.11) is highly correlated (r=0.95) with measured crypt height (Table IV.4). A comparison of Tables IV.3 and IV.1 and Figures IV.11 and IV.1 shows that for all 4 groups these two variables paralleled each other. In the atrophic colonic segments of the bypass controls, both exhibited the same degree of reduction. One week after surgery, morphometric values of the proximal segment decreased to 51% of controls while cell counts decreased to 59%. The corresponding data were 71% and 72% by week 2 and 60% and 63% by week 12.

In the inflamed controls, the initial response to the injurious agent was of similar magnitude to that of crypt height expressed in micrometers. This was indicated by a 2-fold increase in the number of cells per crypt column (70 for IFCN vs 32 for controls). As before, peak levels were observed at week 1 in the descending colon and at week 3 in the transverse.

The reductive trend exhibited by the inflamed-operated animals was also highly correlative between crypt height expressed in micrometers and as number of cells. Again, for the first four weeks (distal colon), IFBP values fell between those of BPCN and IFCN. At week 1, for example, mean cell counts were 70 for inflamed controls, 54 for inflamed-bypass and 29 for bypass controls; the IFBP values

Table IV.3. Crypt Column Height in Number of Cells

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	31.6±0.9[1] (12)[2]	32.2±1.1 (6)	18.7±0.4 (6)[4]	27.8±03.6 (6)	33.8±1.0 (6)	22.6±0.9 (6)[3]	19.9±0.3 (6)	33.1±1.7 ([illegible])	19.4±0.6 (6)[3]	22.1±1.4 (6)	32.0±0.7 (7)	19.6±0.4 (6)[3]	19.8±0.8 (6)
Transverse	32.2±0.6 (12)	42.3±4.5 (6)	26.3±0.3 (6)[4]	37.7±4.1 (6)	40.8±1.3 (6)	25.4±0.6 (6)[3]	28.5±0.3 (6)	47.8±2.1 (8)	26.1±0.5 (6)[3]	31.1±1.2 (6)	42.1±1.8 (8)	25.8±0.5 (6)[3]	29.5±0.8 (6)
Descending	32.3±0.3 (12)	70.2±2.9 (6)	29.2±1.0 (6)[4]	54.6±4.8 (6)	63.1±3.2 (8)	32.1±3.9 (7)[4]	43.5±2.2 (7)	50.2±2.8 (7)	32.5±0.8 (6)[3]	38.6±1.4	44.4±1.5 (6)	29.9±0.6 (6)[3]	37.9±2.7 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	28.8±1.3 (6)	16.9±0.5 (6)[3]	24.8±4.2 (5)	32.0±0.5 (6)	20.7±0.7 (6)[3]	21.8±0.9 (6)	32.9±0.9 (7)	19.4±0.79 (6)[3]	19.8±0.8 (6)	33.4±1.0 (6)	20.0±0.6 (6)[3]	21.9±1.1 (6)
	34.4±2.3 (6)	25.5±0.8 (6)[4]	34.4±4.8 (5)	32.5±0.7 (6)	26.7±0.5 (6)[4]	29.1±0.9	31.6±0.5 (7)	25.9±0.47 (6)[3]	26.5±0.5 (6)	32.1±0.3 (9)	25.9±0.3 (6)[3]	26.9±0.6 (6)
	39.1±1.8 (6)	31.3±0.5 (6)[3]	39.9±4.4 (5)	35.9±1.7 (6)	30.8±1.3 (7)[3]	34.2±1.7 (6)	32.2±0.2 (6)	29.4±0.68 (6)[3]	30.2±0.6 (7)	33.5±0.7 (9)	30.4±0.6 (6)[3]	34.0±1.1 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	9.09	<0.001	<0.05

** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.
[5] No significant differences between the 3 groups.
[6] Significantly different from each other.
[7] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

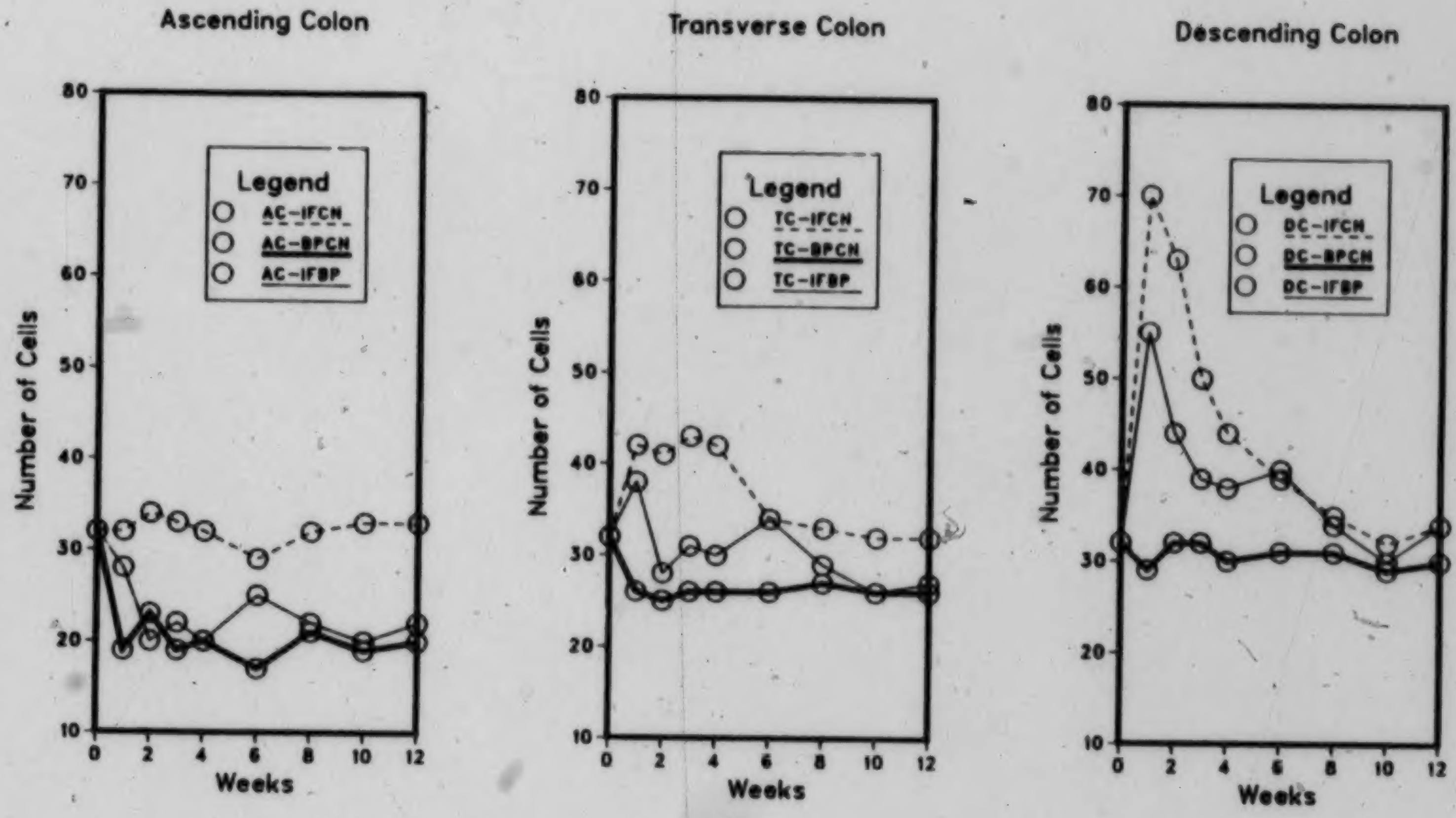


Figure IV.11. Crypt Column Height in Number of Cells (Control: x=0,y)

Table IV.4. Pearson Correlation Coefficients (r) Between Selected Variables[1]

	A	Z	AZ	B	B_1	C	FBMEAN	FHMB	PZ	TPZB	F_1	F_1/A	MEAND	MEANE	X	Y
A	1.00															
Z	0.71	1.00														
AZ	0.97	0.84	1.00													
B	0.95	0.68	0.91	1.00												
B_1	0.94	0.67	0.90	0.99	1.00											
C	0.95	0.67	0.91	0.99	0.99	1.00										
FBMEAN	0.62	0.52	0.61	0.68	0.68	0.68	1.00									
FHMB	-0.01*	0.07*	0.01*	-0.02*	-0.02*	-0.01*	0.65	1.00								
PZ	0.85	0.63	0.83	0.84	0.83	0.84	0.71	0.12**	1.00							
TPZB[a]	0.13*	0.12*	0.15*	0.05*	0.05*	0.05*	0.26	0.32	0.55	1.00						
F_1	0.64	0.53	0.64	0.64	0.63	0.63	0.46	0.14	0.66	0.20	1.00					
F_1/A	0.28	0.35	0.30	0.32	0.31	0.31	0.27	0.18	0.37	0.16	0.89	1.00				
MEAND	0.76	0.67	0.78	0.77	0.77	0.77	0.45	-0.11	0.66	0.04*	0.48	0.22	1.00			
MEANE	0.68	0.83	0.76	0.65	0.65	0.64	0.42	-0.04*	0.55	0.06*	0.46	0.27	0.80	1.00		
X	0.65	0.59	0.68	0.66	0.66	0.67	0.47	0.01*	0.64	0.12**	0.44	0.23	0.69	0.52	1.00	
Y	0.64	0.87	0.75	0.65	0.65	0.65	0.53	0.10**	0.58	0.12**	0.47	0.30	0.66	0.81	0.66	1.00

[1] $P<0.01$ except for coefficients with stars (*).

* Not significant.

** $P<0.05$.

[a] TPZB = Size of proliferative zone (100 x PZ/B); for explanation of other variables, see Table IV.2.

significant against the other two.

B. Total Crypt Cell Population

The total number of cells per crypt estimated by the product of column length and column count is presented in Table IV.5 and Figure IV.12. Results follow those for column length (r=0.97) and measured crypt height (r=0.91) very closely. In the ascending colon, bypass produced similar values in both operated groups and again, these were in general significantly lower than in the control and inflamed only animals. At the end of the first postoperative week, crypt cellularity for the inflamed-bypass rats was between those of bypass and inflamed controls. However, from week 2 (week 6 excepted), they had fallen to bypass control levels, which was equivalent to about half of normal controls, and remained virtually constant up to 3 months after surgery.

As already seen, the isolated transverse colon underwent less atrophy than did the proximal segment in the bypass-operated animals (BPCN). Nevertheless, within 7 days there was a highly significant cell loss of 41% of controls that had leveled off to about 63% by weeks 10 to 12.

Experimental colitis induced a significant hyperplasia of 220% of controls in the distal colon and 144% in the transverse within 1 week for the inflamed controls. Thereafter, crypt cellularity for the descending colon fell steadily while still keeping significantly higher values than normal, that is, 216%, 163%, 148% and 118% of control

Table IV.5. Total Number of Cells per Crypt

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	606.9±24.1[1] (12)[2]	645.0±30.6 (6)	231.4±6.3[4] (6)	450.1±76.4 (6)	662.2±19.8 (6)	295.6±12.45 (6)[4]	245.7±16.7 (6)	641.0±30.3 (7)	250.8±6.3 (6)[4]	291.1±25.3 (6)	619.7±13.8 (7)	248.9±6.4 (5)[4]	264.1±17.2 (6)
Transverse	606.7±20.8 (12)	849.3±109.4 (6)	360.7±7.9 (6)[4]	682.6±104.9 (6)	806.8±42.6 (6)	347.5±18.5 (6)[4]	412.8±8.0 (6)	890.0±50.3 (8)	387.9±16.6 (6)[4]	463.8±27.3 (6)	887.1±37.6 (8)	371.3±20.4 (6)[4]	451.9±28.2 (6)
Descending	677.4±18.6 (12)	1493.3±76.4 (6)	462.0±40.2 (6)[4]	1121.3±130.8 (6)	1460.0±77.3 (6)	604.6±26.6 (7)[4]	870.6±70.2 (7)	1101.4±65.5 (7)	698.6±34.8 (6)[4]	853.6±52.1 (6)	1002.1±38.1 (6)	585.9±27.5 (6)[4]	745.2±63.3 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	538.5±25.6 (6)	194.1±22.7 (6)[4]	406.9±104.5 (5)	663.1±10.6 (6)	282.4±18.7 (6)[4]	302.6±11.6 (6)	628.9±19.2 (6)	247.3±10.5 (6)[4]	269.0±11.9 (6)	666.8±13.3 (6)	232.6±12.2 (6)[4]	314.6±23.6 (6)
	592.8±52.0 (6)	350.0±15.6 (6)[4]	564.1±113.1 (5)	651.7±20.7 (6)	420.5±13.6 (6)[4]	419.8±25.6 (6)	609.7±11.9 (7)	360.5±12.6 (6)[4]	374.7±19.9 (6)	614.5±10.1 (9)	402.8±27.2 (6)[4]	385.9±19.5 (6)
	800.8±39.0 (6)	583.4±31.3 (6)[4]	797.6±98.9 (6)	752.4±40.4 (6)	631.8±48.2 (6)[4]	621.2±33.5 (10)	664.0±11.5 (6)	562.6±16.5 (6)[4]	560.1±30.9 (7)	702.2±25.7 (9)	564.9±24.8 (6)[4]	697.9±31.0 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	7.10	<0.001	<0.05

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.

[5] No significant differences between the 3 groups.

[6] Significantly different from each other.

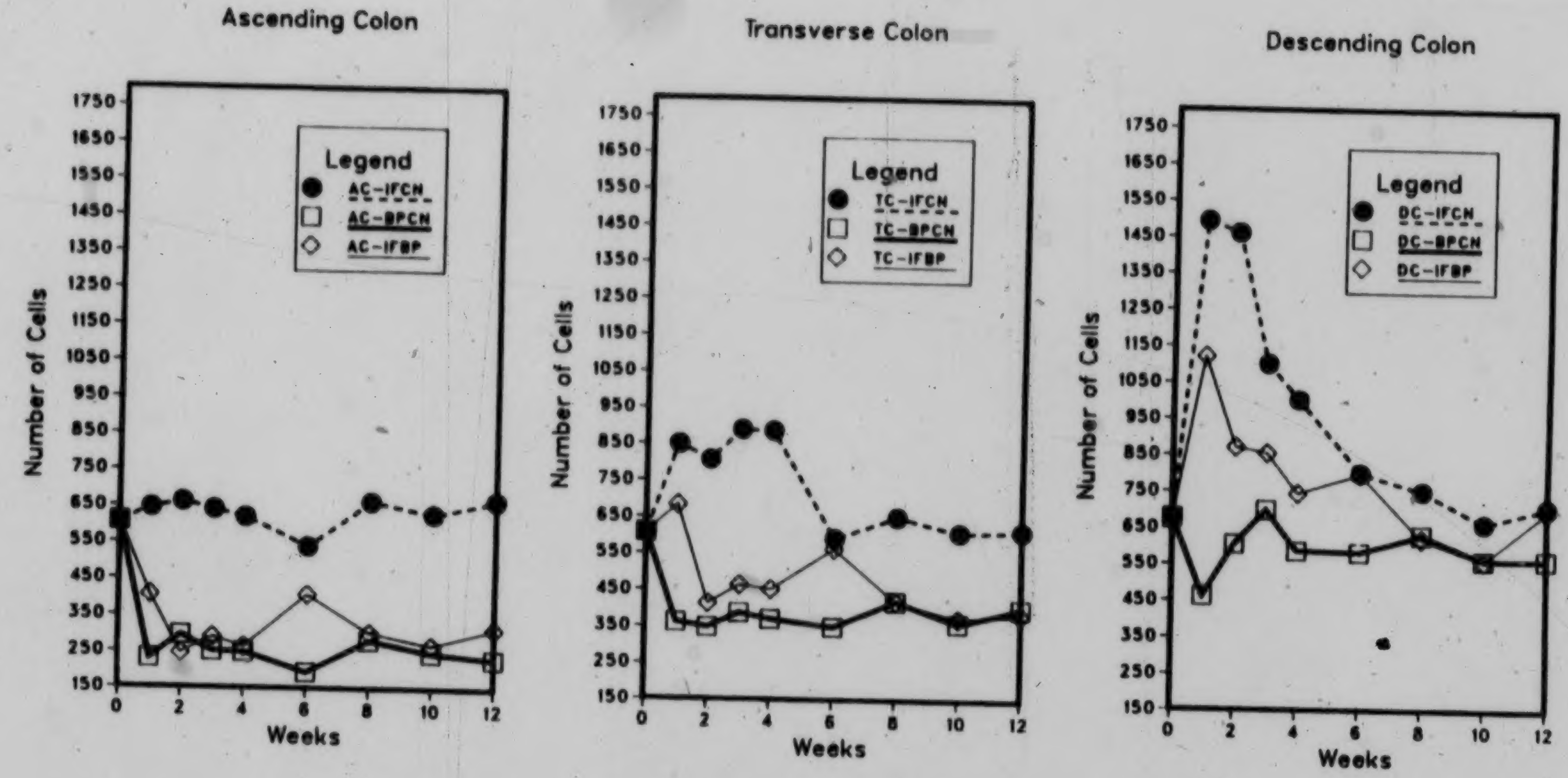


Figure IV.12. Total Number of Cells per Crypt (Control: x=0,y)

values at weeks 2, 3, 4, and 6, respectively. By week 8, however, the glands were again normoplastic. The transverse colon in slight contrast, achieved peak levels about 1.5 times that of controls at week 3; 2 weeks later than in the distal colon. Conversely, natural reversion to normal values occurred 2 weeks earlier at week 6. In the case of the ascending colon, inflammation was observed to have no significant effect on total crypt cell population, as shown in Table IV.2.

The hyperplasia induced in the middle and distal segments was affected by creation of an end-to-side anastomosis. Bowel rest induced a protective effect from experimental colitis by decreasing the extremely high inflamed values seen in the inflamed controls. This reduction was greater in the tranverse region than in the distal - with increasing distance from the anastomotic site. Within 1 week, inflamed values had dropped 25% (1493 cells for IFCN vs 1121 cells for IFBP) in the descending colon and 20% (849 vs 683) in the transverse; nevertheless, at 2, 3 and 4 weeks respectively, they had decreased by 40% and 48%; 22% and 49%, and 26% and 49%. Therefore, the number of cells per crypt returned to, or fell below, normal values in the transverse long before it did in the distal colon; in the former by week 2 and in the latter by week 4.

C. Crypt Column Count

The results for column count are shown in Table IV.6 and Figures IV.13 and IV.14. One week after bypass was established, the mean number of cell columns per crypt circumference for the ascending colon was markedly reduced by 36% of controls; low values persisting until week 12. The mean column count for animals with a bypass alone (BPCN) ranged from 11 to 14 cells, compared with 12 to 16 cells in those that were inflamed before surgery (IFBP), 19 to 21 cells in inflamed only (IFCN) animals and 19 cells in the non-operated controls. As Table IV.2 shows, significant differences existed between the first two groups and the last two. Furthermore, as the results of Scheffe's multiple comparison (segment x group x week) indicate, values for the operated groups were similar and at a significantly lower level than in the rats that were inflamed only.

The reduction in column count diminished towards the distal colon and was, therefore, less intense in the transverse colon than in the proximal. Crypts of the latter were narrower with constant values of 11 to 14 cells compared to 14 to 16 in the former. Similar to the pattern observed in the proximal segment, in the transverse colon, lower mean column counts for the operated groups were statistical significant from inflamed controls except at weeks 1 and 6.

Descending colonic comparison between the four groups (Table IV.2), showed values for bypass-operated (BPCN)

Table IV.6. Number of Columns per Crypt

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	19.14±29[1] (12)[2]	19.98±0.28 (6)	12.30±0.09 (6)[3]	15.53±0.57 (6)	19.60±0.17 (6)	13.06±0.35 (6)[3]	12.24±0.65 (6)	19.39±0.29 (7)	12.92±0.16 (6)[3]	13.17±0.33 (6)	19.37±0.05 (7)	12.73±0.29 (5)[3]	13.35±0.43 (6)
Transverse	18.79±0.40 (12)	19.83±0.62 (6)	13.69±0.20 (6)[4]	17.73±1.17 (6)	19.70±0.55 (6)	13.56±0.47 (6)[3]	14.36±0.26 (6)	20.79±0.39 (8)	14.68±0.46 (6)[3]	14.84±0.37 (6)	21.09±0.37 (8)	14.24±0.56 (6)[3]	15.31±0.64 (6)
Descending	20.79±0.43 (12)	21.21±0.27 (6)	15.72±0.97 (6)[4]	20.35±1.038 (6)	23.05±0.22 (6)	18.62±0.65 (7)[3]	19.75±0.85 (7)	21.74±0.08 (7)	21.42±0.78 (6)[3]	21.95±0.88 (6)	22.56±0.36 (6)	19.56±0.66 (6)[3]	19.58±0.39 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	18.70±0.29 (6)	11.34±0.94 (6)[4]	15.29±1.36 (5)	20.70±0.50 (6)	13.61±0.50 (6)[3]	13.87±0.28 (6)	19.36±0.15 (6)	12.68±0.13 (6)[3]	13.55±0.28 (6)	20.00±0.30 (6)	11.64±0.38 (6)[3]	14.26±0.62 (6)
	17.16±0.55 (6)	13.72±0.36 (6)[3]	16.00±1.01 (5)	20.04±0.32 (6)	15.72±0.37 (6)[3]	14.40±0.87 (6)	19.24±0.19 (7)	13.92±0.42 (6)[3]	14.12±0.63 (6)	19.15±0.23 (9)	15.55±0.94 (6)[3]	14.31±0.43 (6)
	20.54±0.31 (6)	18.59±0.74 (6)[3]	19.70±0.76 (5)	20.77±0.09 (6)	20.15±0.63 (6)[3]	18.16±0.75 (6)	20.61±0.35 (6)	19.15±0.38 (6)[3]	18.51±0.88 (7)	21.09±0.42 (8)	18.65±0.72 (6)[3]	20.51±0.59 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.18	<0.002	<0.05

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

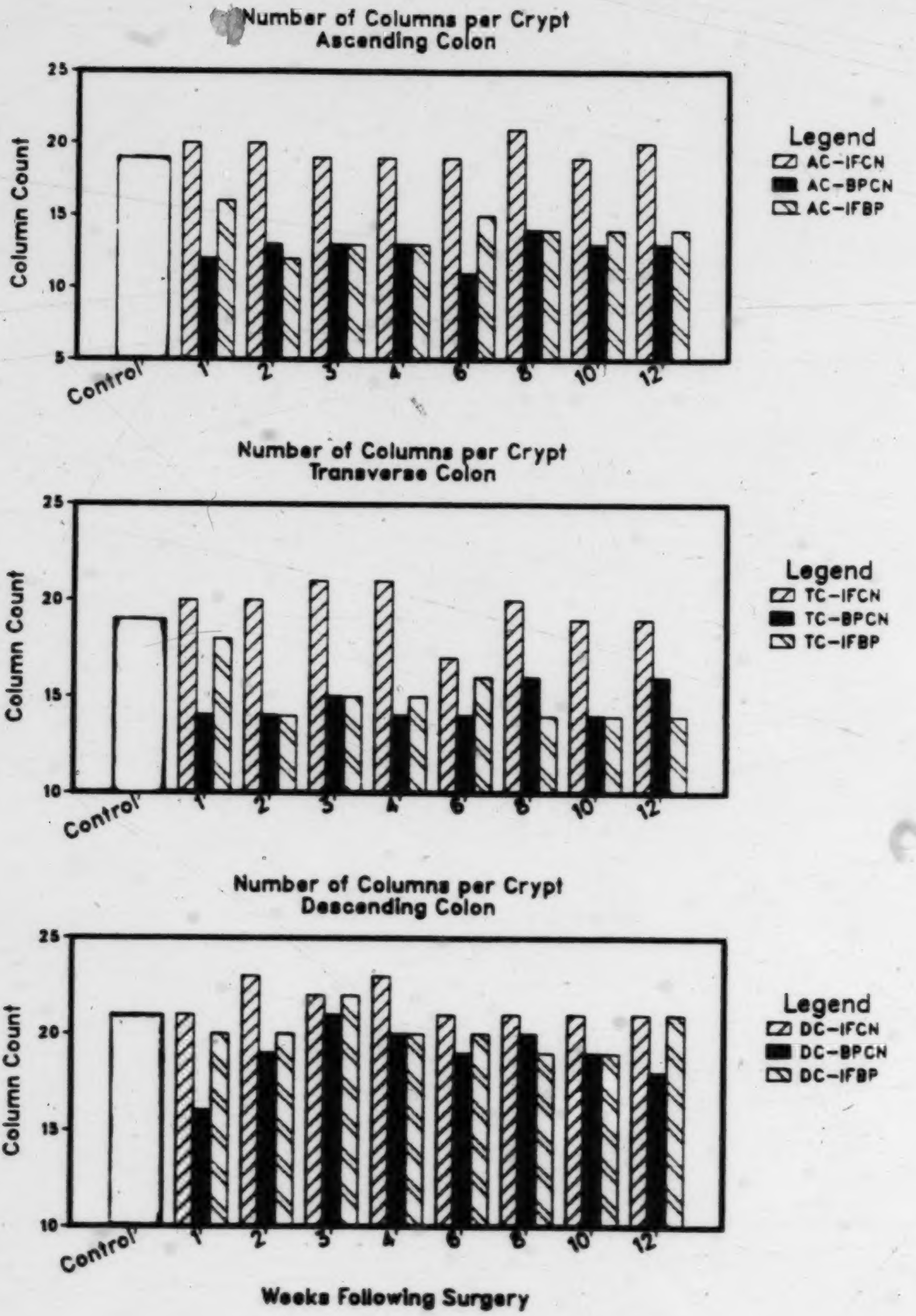
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.

[5] No significant differences between the 3 groups.

[6] Significantly different from each other.

[7] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.



Number of Columns per Crypt
Ascending Colon

Number of Columns per Crypt
Transverse Colon

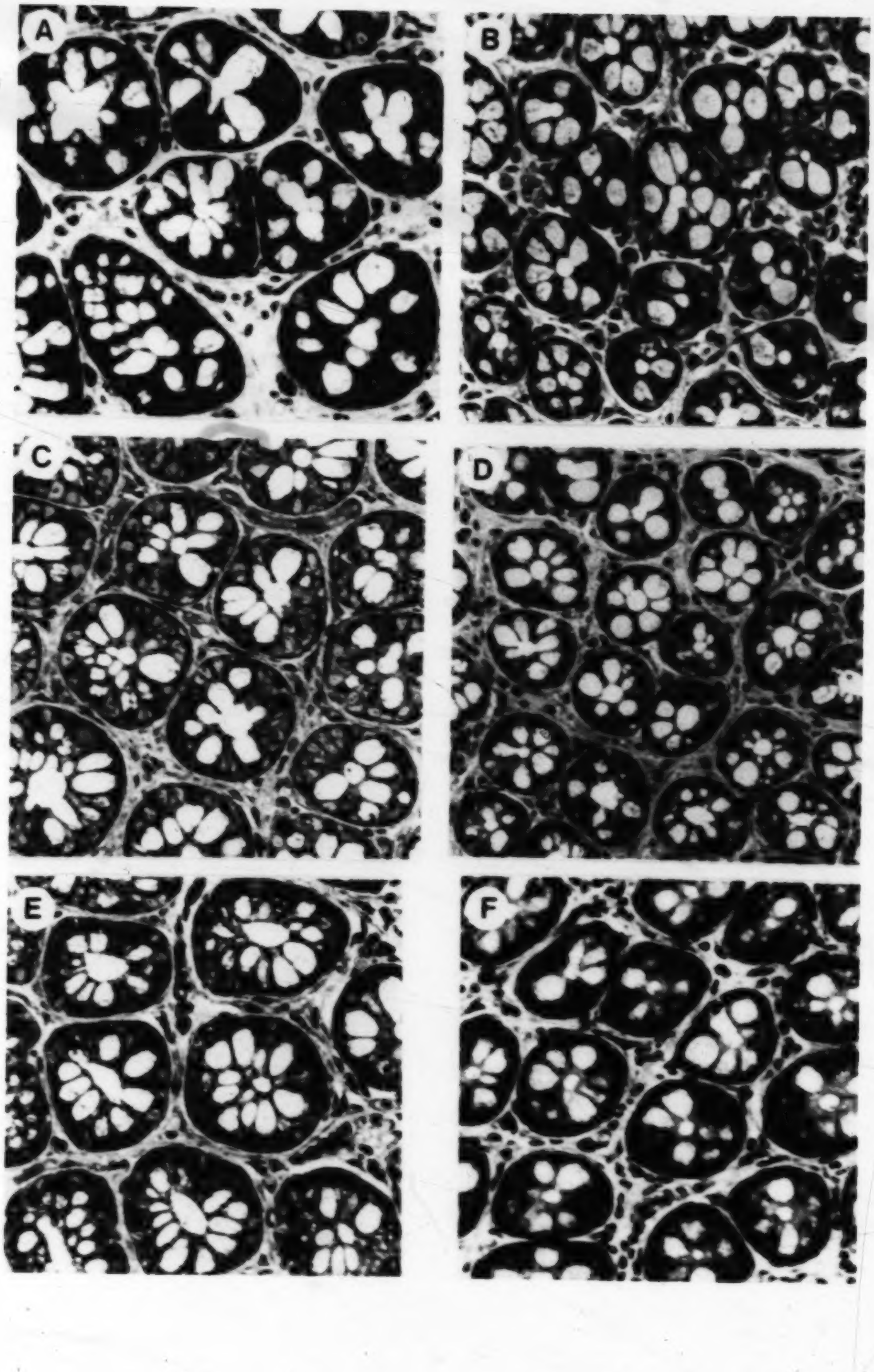
Number of Columns per Crypt
Descending Colon

Figure IV.13. Number of Columns per Crypt

Figure IV.14A,B. Comparison of cross-sectioned crypts between normal (A) and week 8 BPCN (B) ascending colon. The atrophic large bowel shows a significant reduction in column count and crypt width. MBBF, X290.

C,D. As above, but for the normal (C) and defunctioned (D) transverse colon. MBBF, X290.

E,F. Frontal glands of descending colon of non-operated controls (E) and those of bypass controls (F) at 1 week. MBBF, X290.


A
B
C
D
E
F

animals to be significantly lower than controls and inflamed controls. These differences were specifically evident at weeks 1 and 2 and were respectively, 24% and 10% lower than controls and 24% and 17% lower than the inflamed group (IFCN). From weeks 3 to 12, however, there were no significant differences between the three treated groups.

D. Morphometric Diameters

Major Diameters

In accordance with column count, the major diameter of the cross-sectioned crypts (Table IV.7, Figure IV.15) as well as the average major width of longitudinal glands (Table IV.8, Figure IV.16) supported the distance-related response to bypass. In the ascending colon of bypass-operated and inflamed-operated animals, both variables exhibited a reductive trend that ran parallel with a decrease in the number of epithelial cells per crypt circumference (Figure IV.14). Their reduction similarly, reached significance within 7 to 14 days and lasted for the duration of the experimental period.

As with column count, this reduction also slightly decreased towards mid-colon. For bypass controls, crypt diameter in the tranverse colon was markedly reduced, reaching values of 74% and 76% (vs 66% and 73% in the proximal) respectively, within the first week. For the inflamed bypass rats, these figures were 93% and 95%.

Table IV.7. The Major Diameter of Cross-sectioned Crypts*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	49.86±1.40[1] (12)[2]	49.69±1.11 (6)	33.99±0.26 (6)[5]	37.73±2.22 (6)	58.06±1.61 (6)	39.69±3.19 (6)[5]	37.57±1.05 (6)	52.47±0.83 (7)	37.56±0.76 (6)[5]	36.64±0.91 (6)	54.35±1.04 (7)	37.40±1.18 (5)[5]	38.10±1.20 (6)
Transverse	51.39±1.97 (12)	47.23±2.03 (6)	37.86±1.74 (6)[5]	47.83±1.78 (6)	54.03±2.25 (6)	39.75±1.17 (6)[5]	40.41±1.39 (6)	55.83±2.10 (8)	40.37±1.01 (6)[5]	44.34±3.83 (6)	56.51±1.19 (8)	38.36±1.46 (6)[5]	43.70±1.50 (6)
Descending	53.93±1.95 (12)	52.453±1.68 (6)	37.85±2.25 (6)[4]	55.543±1.38 (6)	61.840±1.85 (6)	47.33±2.50 (7)[5]	51.56±2.71 (7)	59.07±1.00 (7)	53.69±2.50 (6)[6]	55.83±2.51 (6)	61.36±6.57 (6)	52.04±2.56 (6)[4]	49.26±2.43 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	51.79±1.51 (6)	33.37±1.14 (6)[5]	39.67±3.87 (5)	56.40±1.71 (6)	38.94±0.57 (6)[5]	40.20±0.62 (6)	52.19±1.10 (6)	35.95±0.60 (6)[5]	35.89±1.31 (6)	54.84±0.60 (6)	33.78±0.74 (6)[5]	40.78±1.70 (6)
	46.00±1.46 (6)	40.13±1.02 (6)[5]	42.92±2.13 (5)	51.98±1.62 (6)	43.60±0.85 (6)[4]	40.92±2.15 (6)	54.60±2.42 (7)	39.65±1.44 (6)[5]	39.84±1.60 (6)	52.73±1.29 (9)	42.10±2.30 (6)[5]	47.50±5.11 (6)
	52.25±2.86 (6)	49.55±1.95 (6)[5]	51.12±1.96 (5)	53.78±0.20 (6)	53.22±1.84 (6)[5]	49.09±1.85 (6)	52.53±1.27 (6)	46.83±1.47 (6)[5]	42.28±2.08 (7)	52.31±1.94 (9)	48.76±2.01 (6)[5]	54.19±1.71 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	1.32	=0.15 [3]NS	<0.05

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* The distance from basement membrane to basement membrane (micrometers).
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.
[3] NS = Not significant.

For Scheffe's Multiple Comparisons:
[4] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[5] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.
[6] No significant differences between the 3 groups.
[7] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.
[8] IF/BP and IF/CN are significantly different from each other but not from BP/CN.

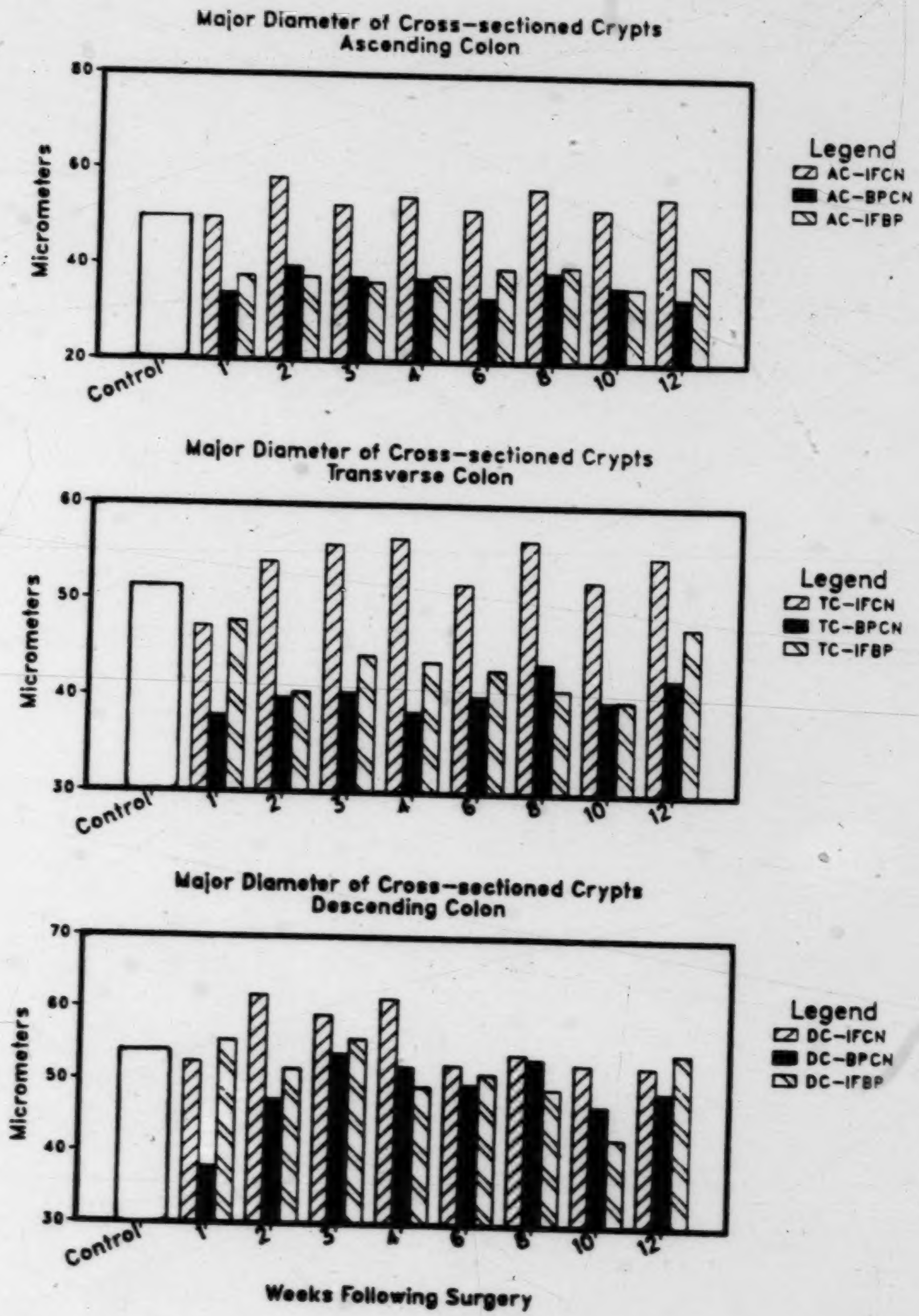

Figure IV.15. The Major Diameter of Cross-sectioned Crypts

Table IV.8. Average Major Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week** IF/CN	One Week** BP/CN	One Week** IF/BP	Two Weeks IF/CN	Two Weeks BP/CN	Two Weeks IF/BP	Three Weeks IF/CN	Three Weeks BP/CN	Three Weeks IF/BP	Four Weeks IF/CN	Four Weeks BP/CN	Four Weeks IF/BP
Colonic Segment	Control	IF/CN	One Week** BP/CN	IF/BP	IF/CN	Two Weeks BP/CN	IF/BP	IF/CN	Three Weeks BP/CN	IF/BP	IF/CN	Four Weeks BP/CN	IF/BP
Ascending	48.0±2.0[1] (12)[2]	43.4±1.8 (6)	35.2±2.1 (6)[3]	35.5±2.0 (6)	58.1±1.1 (6)	37.2±0.9 (6)[3]	35.7±1.0 (6)	52.9±1.4 (7)	36.7±1.5 (6)[3]	36.2±1.3 (6)	51.8±1.1 (7)	38.4±1.8 (6)[3]	36.7±1.6 (6)
Transverse	46.8±2.1 (12)	42.1±2.3 (6)	35.5±1.4 (6)[6]	44.4±2.0 (6)	54.6±2.8 (6)	36.6±1.2 (6)[3]	40.3±1.5 (6)	56.0±2.9 (8)	38.6±0.7 (6)[3]	40.6±2.3 (6)	53.9±1.8 (8)	39.6±1.9 (6)[3]	43.3±2.3 (6)
Descending	50.5±1.9 (12)	57.05±1.9 (6)	41:8±2.8 (6)[5]	52.3±1.9 (6)	65.7±2.6 (6)	48.7±2.2 (7)[5]	58.01±2.5 (7)	60.3±2.3 (7)	60.1±4.9 (6)[4]	63.2±3.3 (6)	56.1±1.9 (6)	49.5±2.6 (6)[5]	54.7±3.0 (6)

Six Weeks IF/CN	Six Weeks BP/CN	Six Weeks IF/BP	Eight Weeks IF/CN	Eight Weeks BP/CN	Eight Weeks IF/BP	Ten Weeks IF/CN	Ten Weeks BP/CN	Ten Weeks IF/BP	Twelve Weeks IF/CN	Twelve Weeks BP/CN	Twelve Weeks IF/BP
47.2±1.8 (6)	31.8±1.6 (6)[3]	37.5±3.6 (5)	49.7±1.7 (6)	38.2±1.2 (6)[3]	37.7±1.1 (6)	45.7±1.9 (7)	32.2±0.7 (6)[3]	33.0±1.0 (6)	50.1±1.4 (6)	35.5±1.6 (6)[3]	38.6±1.4 (6)
44.5±1.3 (6)	41.1±1.8 (6)[4]	43.2±2.3 (5)	50.7±1.9 (6)	46.4±2.1 (6)[6]	40.8±1.7 (6)	48.7±0.7 (7)	38.8±1.9 (6)[3]	39.3±2.1 (6)	51.8±1.6 (9)	42.5±3.2 (6)[6]	40.5±1.9 (6)
50.9±1.2 (6)	58.9±2.2 (6)[5]	48.4±1.1 (5)	52.0±1.1 (6)	52.4±2.0 (7)[4]	47.8±2.0 (6)	50.3±2.0 (6)	48.8±0.9 (6)[5]	50.3±1.1 (7)	55.7±1.4 (9)	53.2±3.5 (6)[4]	54.8±2.3 (6)

	One-way Analysis of Variance Statistics F	One-way Analysis of Variance Statistics P	Scheffe's Multiple Comparison Test P
Segment x Group x Week	2.65	<0.001	<0.05

* The average of apical, medial and basal major crypt widths (micrometers).

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[4] No significant differences between the 3 groups.

[5] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

[6] IF/BP and IF/CN are significantly different from each other but not from BP/CN.

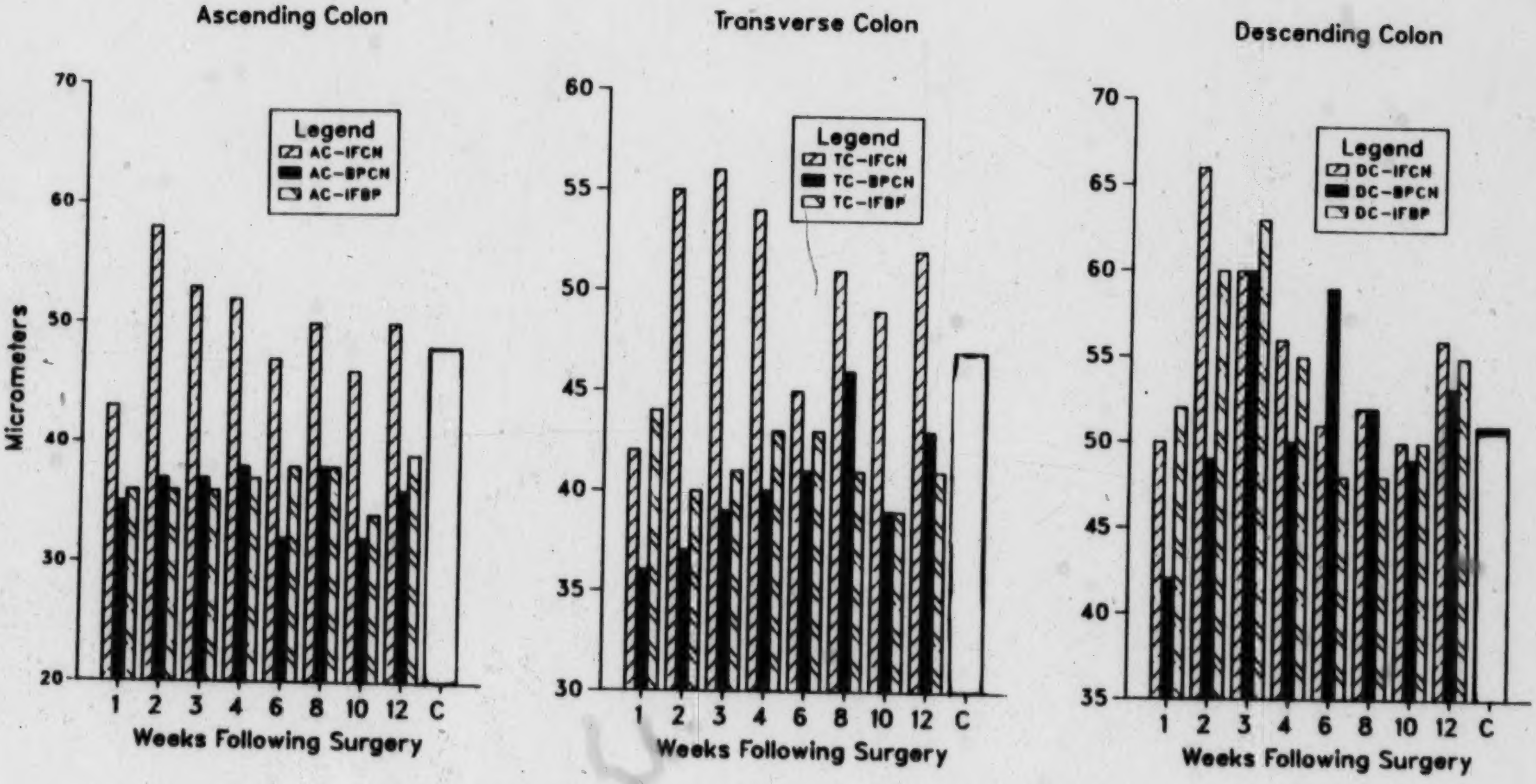

Figure IV.16. Average Major Crypt Width of Longitudinal Glands

Thereafter, the morphometric data for both groups were generally at a significantly lower level than those for non-operated and inflamed controls. Again, in the distal colon, response patterns were identical to those of column count.

The influence of inflammation on these parameters was very similar to that observed in column count; in the 3 segments and for the 3 groups, with corresponding insignificant increases for some of the weekly intervals.

Results for width parameters, column count included, were further substantiated by the data for the number of transversely cut crypts per unit area (Table IV.9). While in the atrophic large bowel data for these two sets of variables were inversely related, no changes were seen in the number of cross-sectioned glands in the absence of clear-cut differences in width parameters between any of the four groups. There were also no differences in interglandular distance (Table IV.10) between the three segments of all groups.

Minor Diameters

The width from cell surface to cell surface of longitudinal crypts obtained from the average of apical, medial and basal measurements (Appendix 6) is shown in Table IV.11. That for transverse glands is given in Table IV.12. Inflammation was observed to have a significant effect on the average minor crypt width of transverse and descending

Table IV.9. The Number of Transversely Sectioned Crypts Per Unit Area*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	10.70±0.65 (9)	19.50±0.76 (6)	25.94±0.34 (6)	20.10±2.39 (5)	10.11±0.49 (6)	21.44±1.66 (6	21.89±1.05 (6)	10.62±0.39 (7)	20.75±0.98 (6)	20.56±0.68 (6)	11.33±0.93 (7)	21.13±0.60 (5)	19.20±0.71 (5)
Transverse	56.67±3.87 (12)	67.06±3.34 (6)	73.06±6.78 (6)	63.22±6.19 (6)	50.44±4.11 (6)	68.50±3.82 (6)	83.94±2.78 (6)	48.13±2.93 (8)	66.56±0.99 (6)	73.17±3.08 (6)	49.88±3.55 (8)	67.94±4.25 (6)	70.61±4.47 (6)
Descending	48.85±2.35 (11)	43.61±2.67 (6)	68.33±8.08 (6)	52.943±4.22 (6)	37.00±2.39 (6)	58.81±4.38 (7)	53.52±3.53 (7)	41.55±1.45 (7)	48.89±4.82 (6)	44.67±3.94 (6)	45.10±3.96 (6)	57.11±6.43 (6)	50.56±3.44 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		13.42±0.24 (6)	27.87±0.77 (5)	17.60±2.87 (5)	11.33±0.54 (6)	14.00 (1)	17.93±2.36 (5)	12.91±1.06 (7)	21.50±0.74 (6)	20.06±0.88 (6)	11.67±0.73 (6)	26.17±1.68 (6)	20.17±1.99 (6)
		63.94±3.41 (6)	81.28±3.19 (6)	68.00±6.26 (5)	49.56±1.42 (6)	60.51±1.57 (6)	70.22±4.04 (6)	54.91±2.93 (7)	69.44±2.06 (6)	75.61±5.09 (6)	47.44±3.23 (9)	72.17±2.71 (4)	68.37±7.66 (6)
		44.60±2.45 (5)	49.33±1.13 (6)[1]	51.63±4.10 (5)	45.50±0.51 (6)	40.00±1.42 (6)[1]	53.39±3.51 (6)	49.33±1.40 (6)	53.17±1.97 (6)[1]	59.48±4.87 (7)	52.42±1.97 (8)	54.14±4.35 (6)[1]	46.22±2.04 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	1.98	<0.01	<0.05 [a]NC

* The number of glands seen in 3 random areas (frontal sections) using the X40 objective for the ascending colon and the X10 lens for transverse and descending colonic segments (see Materials and Methods).

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[a] NC = Not calculated for Segment x Group x Week (See Table IV.2).

Table IV.10. Distance Between Crypt*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	13.93±0.79[1] (12)[2]	13.85±1.25 (6)	12.48±0.92 (6)	12.88±1.13 (6)	11.56±2.80 (6)	9.83±0.54 (6)	13.25±1.16 (6)	7.52±0.91 (7)	11.56±0.58 (6)	10.61±1.13 (6)	9.57±1.13 (6)	9.97±0.52 (5)	12.35±1.02 (4)
Transverse	11.24±0.42 (12)	13.32±0.97 (6)	12.08±0.71 (6)	11.06±1.22 (6)	14.29±1.23 (6)	11.06±0.75 (6)	11.11±0.59 (6)	12.99±1.18 (8)	11.31±0.98 (6)	10.71±0.33 (6)	12.00±0.94 (8)	11.51±1.18 (6)	12.85±0.87 (6)
Descending	12.27±0.59 (12)	13.95±1.07 (6)	14.42±1.51 (6)	11.99±1.04 (6)	11.58±0.56 (6)	12.57±1.11 (7)	12.17±0.67 (7)	9.90±0.76 (7)	11.92±0.84 (6)	11.15±1.24 (6)	11.25±0.93 (6)	12.18±0.63 (6)	10.99±1.04 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	9.64±0.77 (6)	11.68±1.05 (6)	12.85±0.90 (5)	12.06±0.97 (6)	11.88±0.97 (6)	13.39±0.96 (6)	11.04±0.58 (7)	9.85±0.52 (6)	11.86±1.17 (6)	12.72±0.90 (6)	10.61±0.65 (6)	11.56±0.91 (6)
	11.04±0.47 (6)	9.24±0.68 (6)	10.27±1.09 (5)	12.98±1.12 (6)	11.65±0.50 (6)	11.65±0.55 (6)	12.35±0.75 (7)	12.04±1.11 (6)	11.24±0.27 (6)	13.30±0.54 (9)	10.55±1.63 (6)	11.15±0.10 (6)
	12.19±1.33 (5)	12.05±0.65 (6)	10.71±2.25 (5)	11.07±0.74 (6)	13.12±0.64 (6)	11.10±1.03 (6)	14.09±2.64 (6)	10.50±0.57 (6)	10.40±0.70 (7)	12.56±1.26 (8)	10.39±0.52 (6)	11.06±1.44 (6)

One way Analysis of Variance Statistics

Segment x Group x Week	F=0.71	P=0.850 NS[3]

* Linear distance along the lamina propria between 15 consecutive, axial glands (arbitrary units).
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

[3] NS = Not significant.

Table IV.11. Average Minor Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	9.10±0.61[1] (12)[2]	7.03±0.33 (6)	6.95±0.58 (6)[3]	7.52±0.80 (6)	10.52±0.38 (6)	8.01±0.43 (6)[6]	6.75±0.28 (6)	10.92±0.29 (7)	7.83±0.29 (6)[6]	6.73±0.29 (6)	10.17±0.41 (7)	7.71±0.48 (5)[6]	6.90±0.53 (6)
Transverse	10.31±0.31 (12)	9.87±0.38 (6)	9.07±0.34 (6)[8]	14.80±0.75 (6)	19.93±0.91 (6)	9.07±0.19 (6)[3]	10.08±0.53 (6)	15.24±1.09 (8)	8.74±0.40 (6)[7]	9.52±0.54 (6)	14.72±0.69 (8)	9.10±0.16 (6)[3]	10.54±0.41 (6)
Descending	10.00±0.68 (12)	14.71±0.59 (6)	9.09±0.59 (6)[4]	15.96±1.12 (6)	18.18±1.04 (6)	10.73±0.49 (7)[3]	14.36±1.26 (7)	16.67±1.09 (7)	13.00±1.30 (6)[7]	14.28±1.15 (6)	13.54±0.77 (6)	10.62±0.49 (6)[4]	11.37±0.82 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	7.54±0.71 (6)	5.88±0.11 (6)[5]	7.33±1.10 (5)	9.77±0.56 (6)	7.33±0.20 (6)[5]	7.08±0.35	8.86±0.44 (7)	6.65±0.40 (6)[5]	5.48±0.30 (6)	9.19±0.43 (6)	6.14±0.22 (6)[5]	7.78±0.40 (6)
	10.71±0.65 (6)	8.95±0.50 (6)[5]	10.06±0.73 (5)	11.78±0.52 (6)	9.74±0.48 (6)[5]	9.17±0.73 (6)	11.06±0.32 (7)	8.68±0.39 (6)[5]	9.29±0.49 (6)	11.67±0.46 (9)	9.16±0.93 (6)[5]	8.32±0.40 (6)
	11.98±1.35 (6)	10.41±0.31 (6)[5]	10.85±0.54 (6)	12.49±0.20 (6)	9.46±6.00 (7)[5]	11.19±0.75 (6)	11.07±0.16 (6)	9.70±0.32 (6)[5]	9.44±0.40 (7)	11.18±0.41 (9)	11.26±0.88 (6)[5]	11.67±0.44 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	3.17	<0.001	<0.05

* The average of apical, medial and basal minor crypt widths (micrometers).
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.
[5] No significant differences between the 3 groups.
[6] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.
[7] IF/BP is significantly different from both BP/CN and IF/CN; the last two being insignificantly different.
[8] IF/BP and IF/CN are significantly different from each other but not from BP/CN.

Table IV.12. The Minor Diameter of Cross-sectioned Crypts*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	10.03±0.37[1] (12)[2]	8.98±0.34 (6)	6.26±0.47 (6)[5]	7.40±0.35 (6)	11.03±0.35 (6)	7.56±0.83 (6)[5]	7.93±0.42 (6)	11.39±0.51 (7)	7.45±0.34 (6)[3]	6.79±0.37 (6)	11.07±0.43 (7)	7.10±0.59(5) [5]	6.86±0.47 (6)
Transverse	10.00±0.26 (12)	12.21±0.96 (6)	9.79±0.71 (6)[10]	17.08±1.02 (6)	14.93±1.18 (6)	9.59±0.08 (6)[4]	10.39±0.78 (6)	16.14±0.93 (8)	9.57±0.44 (6)[3]	9.16±0.48 (6)	16.46±0.71 (8)	7.83±0.49[3] (6)	9.46±0.82 (6)
Descending	13.30±1.10 (12)	16.84±1.51 (6)	9.61±1.16 (6) [4]	18.46±1.82 (6)	15.76±1.07 (6)	9.05±0.63 (7) [5]	10.98±1.00 (7)	16.60±1.05 (7)	11.44±0.81 (6)[4]	10.40±1.22 (6)	19.24±4.45 (6)	10.88±0.46 (6)[3]	9.15±0.83 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	8.73±0.71 (6)	6.20±0.41 (6)[5]	7.10±0.85 (5)	10.71±0.42 (6)	6.98±0.16 (6)[5]	8.01±0.51 (6)	9.83±0.31 (6)	9.32±2.81 (6)[5]	6.01±0.32 (6)	10.00±0.39 (6)	5.39±0.15 (6)[5]	7.95±0.39 (6)
	12.49±0.53 (6)	8.66±0.48 (6)[5]	10.07±0.47 (5)	12.97±0.96 (6)	8.15±0.38 (6)[5]	8.12±0.44 (6)	10.83±0.79 (7)	7.92±0.21 (6)[5]	8.58±0.53 (6)	10.46±0.47 (9)	9.30±0.96 (6)[5]	8.06±0.33 (6)
	13.41±2.99 (6)	9.53±0.34 (6) [5]	9.84±0.89 (5)	11.78±0.23 (6)	8.31±0.64 (6)[5]	9.57±0.60 (6)	11.20±0.82 (6)	8.18±0.48 (6)[5]	7.88±0.79 (7)	12.17±1.20 (8)	8.59±0.53 (6)[5]	10.78±0.50 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	1.60	=0.04	<0.05

*The luminal distance (micrometers).
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.
[5] No significant differences between the 3 groups.
[6] IF/BP and IF/CN are significantly different from each other but not from BP/CN.
[10] The only significant difference is between IF/BP and BP/CN.

colonic regions as shown in Table IV.11 and Figure IV.17. For inflamed controls, the luminal width of the axial glands increased from week 1 to week 4 in the descending colon and from week 2 to week 4 in the transverse. In both regions, it was highest at week 2.

A similar widening was observed in the inflamed-operated animals but, after the first week, measurements were substantially reduced from inflamed controls. For the first, second, third and fourth time intervals mean average crypt width for the distal colon were respectively, 147%, 182%, 167% and 135% above normal whereas those for the inflamed-bypass group were 160%, 144%, 143% and 114%. Accordingly, after a temporary excess, IFBP values followed essentially the same protective trend as in most other variables. This is substantiated by the positive significant correlation between lumenal width (Y) on the one hand and total crypt cell population (AZ), column height (A) and measured crypt height (B) on the other hand (Table IV.4). A similar trend was observed for the minor diameters of cross-sectioned glands (Table IV.12).

For bypass controls, a decreasing tendency was displayed in the ascending and transverse colon. A statistical comparison of these segments between the four groups (Table IV.2) revealed that apical and medial minor diameters of longitudinal crypts (Appendix 6) of the surgically treated groups were generally significantly lower than normal. The descending colon by contrast, did not

Figure IV.17. Week 2 light micrograph of IFCN descending colon, demonstrating colonic cryptitis at center in addition to bifurcating crypts toward the right, and increased inflammatory cell infiltrate. MBBF, X260.

deviate from the control, while exhibiting an irregular response pattern with time.

E. Mucosal Height

Distances from the top (C; Table IV.13) and bottom (B_1; Table IV.14) of crypt base cells to the tip of the surface epithelium were very highly correlated with measured crypt height (B, Table IV.1). This was indicated by a high Pearson correlation coefficient of 0.99 between the three variables (Table IV.4). Interestingly, for the normal colon, the height of the surface epithelium (B_1-B) was estimated as 37.2, 34.7 and 34.9 micrometers for the proximal, middle and distal segments respectively, whereas that for crypt base cells was 17.7, 14.6 and 16 micrometers.

Variables B_1 and C strongly reinforced the distance-related response patterns to bowel rest and inflammation in the colon. This is clearly shown by the curves in Figures IV.18, IV.1, and IV.12 and in the bar charts of Figure IV.19a, b and c. Colonic exclusion from the fecal stream led to a marked mucosal atrophy, whereas contact with 10% acetic acid stimulated considerable hypertrophy. Resting the inflammed bowel demonstrated a tendency to reverse these inflammatory changes to normalcy; by at least four weeks postoperatively.

Table IV.13. Distance from Apex of Crypt Base Cells to Tip of Surface Epithelium (Micrometers)

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	256.6±6.8[1] (12)[2]	251.0±2.4 (6)	141.4±4.7 (6)[4]	216.2±30.1 (6)	302.6±10.0 (6)	187.6±13.5 (6)[3]	170.6±6.1 (6)	277.0±10.0 (7)	166.1±6.7 (6)[3]	193.9±13.1 (6)	281.7±9.3 (7)	153.1±2.6 (5)[3]	172.7±8.4 (6)
Transverse	289.9±5.8 (12)	347.3±34.3 (6)	242.8±8.3 (6)[4]	329.5±30.0 (6)	363.1±13.0 (6)	253.8±8.6 (6)[3]	274.4±9.6 (6)	375.0±20.8 (8)	265.1±4.2 (6)[3]	307.9±7.9 (6)	352.6±11.6 (8)	260.5±9.2 (6)[3]	283.4±13.4 (6)
Descending	265.2±4.0 (12)	497.6±16.3 (6)	243.7±9.5 (6)[4]	417.8±35.1 (6)	489.2±26.5 (6)	278.1±6.1 (7)[6]	363.2±24.6 (7)	445.1±12.8 (7)	280.7±10.5 (6)[3]	318.1±7.4 (6)	383.9±26.4 (6)	268.2±6.5 (6)[3]	306.6±16.6 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		236.5±8.6 (6)	139.9±5.7 (6)[4]	218.2±40.9 (5)	263.0±2.2 (6)	164.4±9.28 (6)[3]	178.1±8.6 (6)	276.9±4.9 (7)	149.3±4.0 (6)[3]	161.3±4.3 (6)	276.9±4.1 (6)	165.2±7.6 (6)[3]	186.1±9.8 (6)
		310.8±22.9 (6)	226.3±9.2 (6)[4]	321.6±36.8 (5)	287.7±8.7 (6)	235.8±8.2 (6)[3]	281.0±12.2 (6)	278.0±6.2 (7)	228.5±9.5 (6)[3]	239.4±7.7 (6)	273.9±9.3 (9)	241.4±6.0 (6)[3]	258.7±5.4 (6)
		333.6±20.7 (6)	264.2±5.7 (6)[4]	349.1±43.1 (5)	302.9±11.5 (6)	236.7±5.4 (7)[3]	291.1±17.2 (6)	270.1±6.9 (6)	243.0±7.3 (6)[3]	249.9±8.0 (7)	269.2±6.6 (9)	252.1±4.6 (6)[3]	266.7±11.4 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	4.47	<0.001	<0.05

** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.
[5] No significant differences between the 3 groups.
[6] Significantly different from each other.
[7] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

Table IV.14. Distance from Crypt Base to Tip of Surface Epithelium (Micrometers)

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	276.1±6.7[1] (12)[2]	270.9±2.9 (6)	157.6±5.2 (6)[4]	232.4 (6)	323.3±10.0 (6)	207.0±14.2 (6)[3]	186.2±6.0 (6)	296.7±9.8 (7)	184.4±7.4 (6)[3]	211.3±13.7 (6)	302.0±9.5 (7)	171.0±2.7 (5)[3]	188.6±8.8 (6)
Transverse	310.0±6.0 (12)	367.2±34.3 (6)	259.4±9.7 (6)[4]	348.1±30.8 (6)	389.3±18.2 (6)	274.5±9.2 (6)[3]	294.9±10.2 (6)	396.1±20.8 (8)	285.3±4.1 (6)[3]	328.9±8.1 (6)	373.4±11.1 (8)	281.3±9.9 (6)[3]	305.1±13.5 (6)
Descending	284.1±4.1	517.9±16.3 (6)	262.5±9.5 (6)[4]	437.7±36.4 (6)	509.8±26.1 (6)	297.7±5.7 (7)[4]	384.8±24.6 (7)	465.9±12.9 (7)	300.8±10.7 (6)[3]	341.3±6.9 (6)	404.9±26.4 (6)	289.7±6.1 (6)[3]	329.8±16.8 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		255.8±8.4 (6)	155.9±5.6 (6)[4]	234.9±41.3 (5)	282.4±1.9 (6)	182.6±9.6 (6)[3]	198.2±8.1 (6)	297.9±4.1 (7)	166.9±3.9 (6)[3]	177.5±4.5 (6)	276.9±4.1 (6)	183.3±8.0 (6)[3]	203.3±10.1 (6)
		330.4±22.5 (6)	246.4±10.0 (6)[4]	341.1±36.5 (5).	308.3±9.2 (6)	255.7±9.0 (6)[5]	301.3±12.6 (6)	298.4±6.2 (7)	247.6±10.1 (6)[5]	258.8±8.4 (6)	273.9±9.3 (9)	261.1±6.4 (6)[5]	279.2±6.1 (6)
		361.5±22.9 (8)	283.8±6.1 (6)[4]	368.4±42.8 (5)	324.2±11.4 (6)	256.2±5.4 (7)[7]	311.1±17.6 (6)	289.8±5.5 (6)	263.6±8.1 (6)[5]	270.7±8.6 (7)	289.0±7.0 (9)	272.7±5.0 (6)[5]	289.1±12.9 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	4.47	<0.001	<0.05

** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.
[5] No significant differences between the 3 groups.
[6] Significantly different from each other.
[7] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

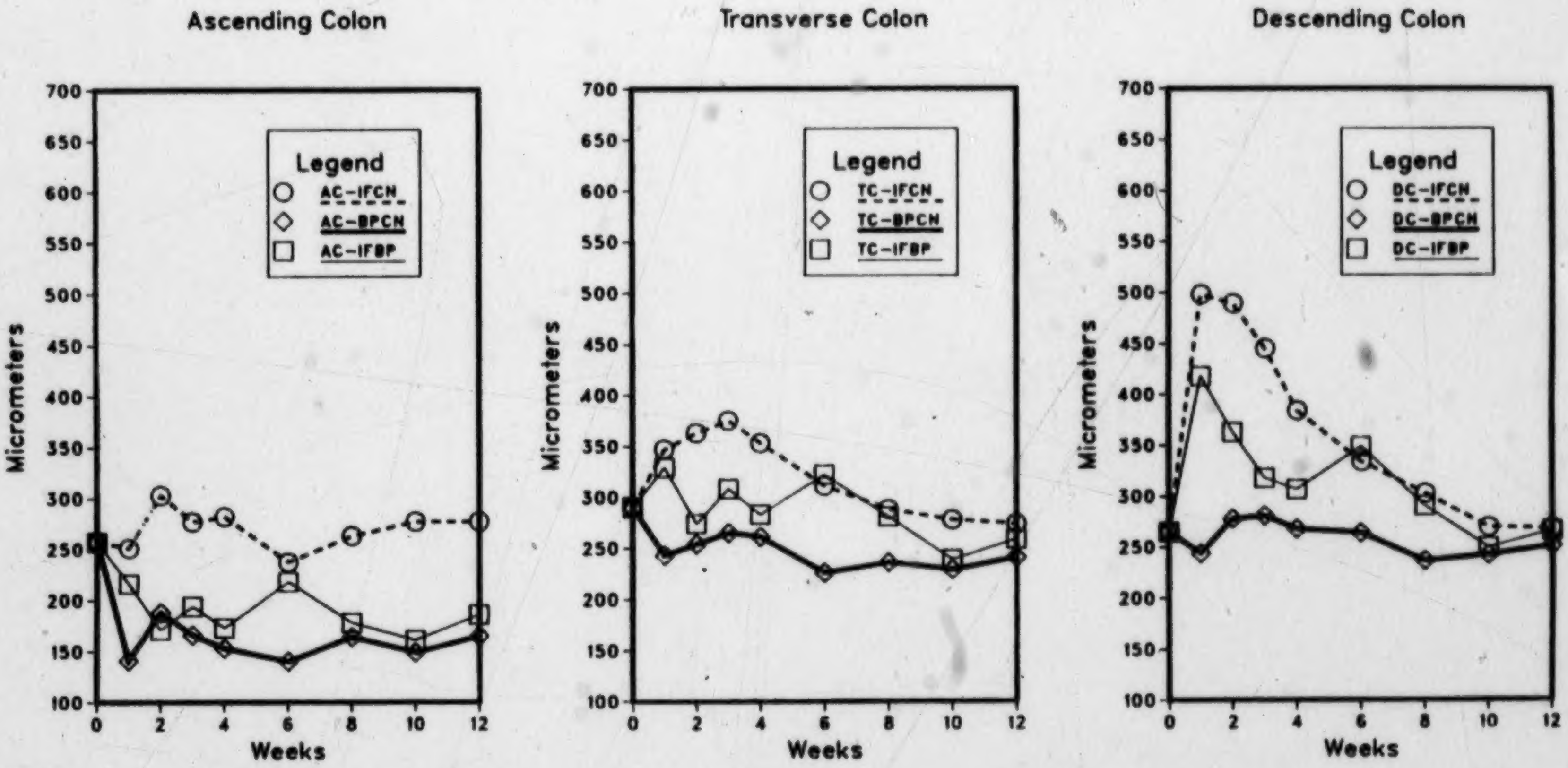

Figure IV.18. Distance from Apex of Crypt Base Cells to Tip of Surface Epithelium (Control: x=0,y)

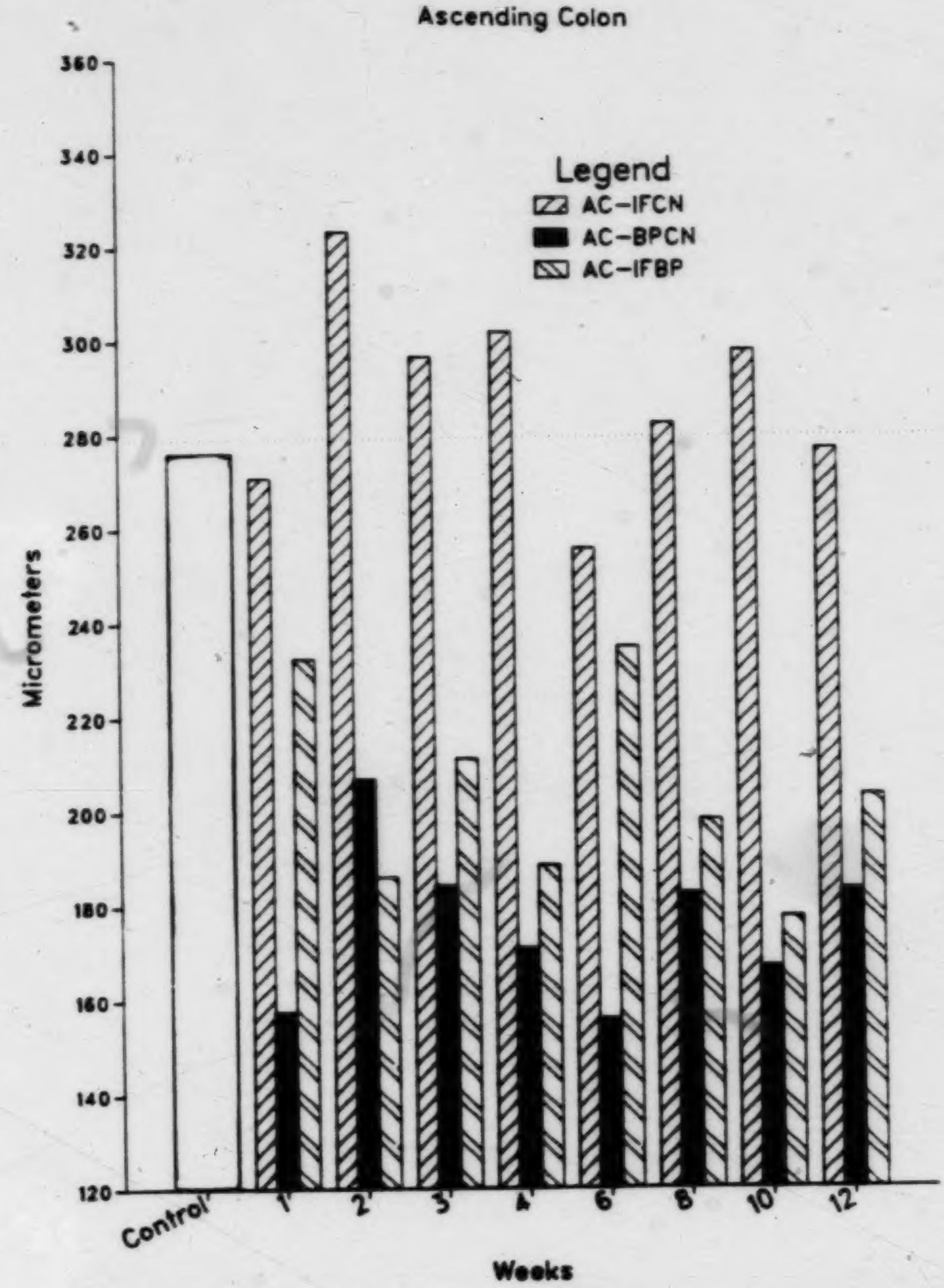

Figure IV.19a. Distance from Crypt Base to Tip of Surface Epithelium

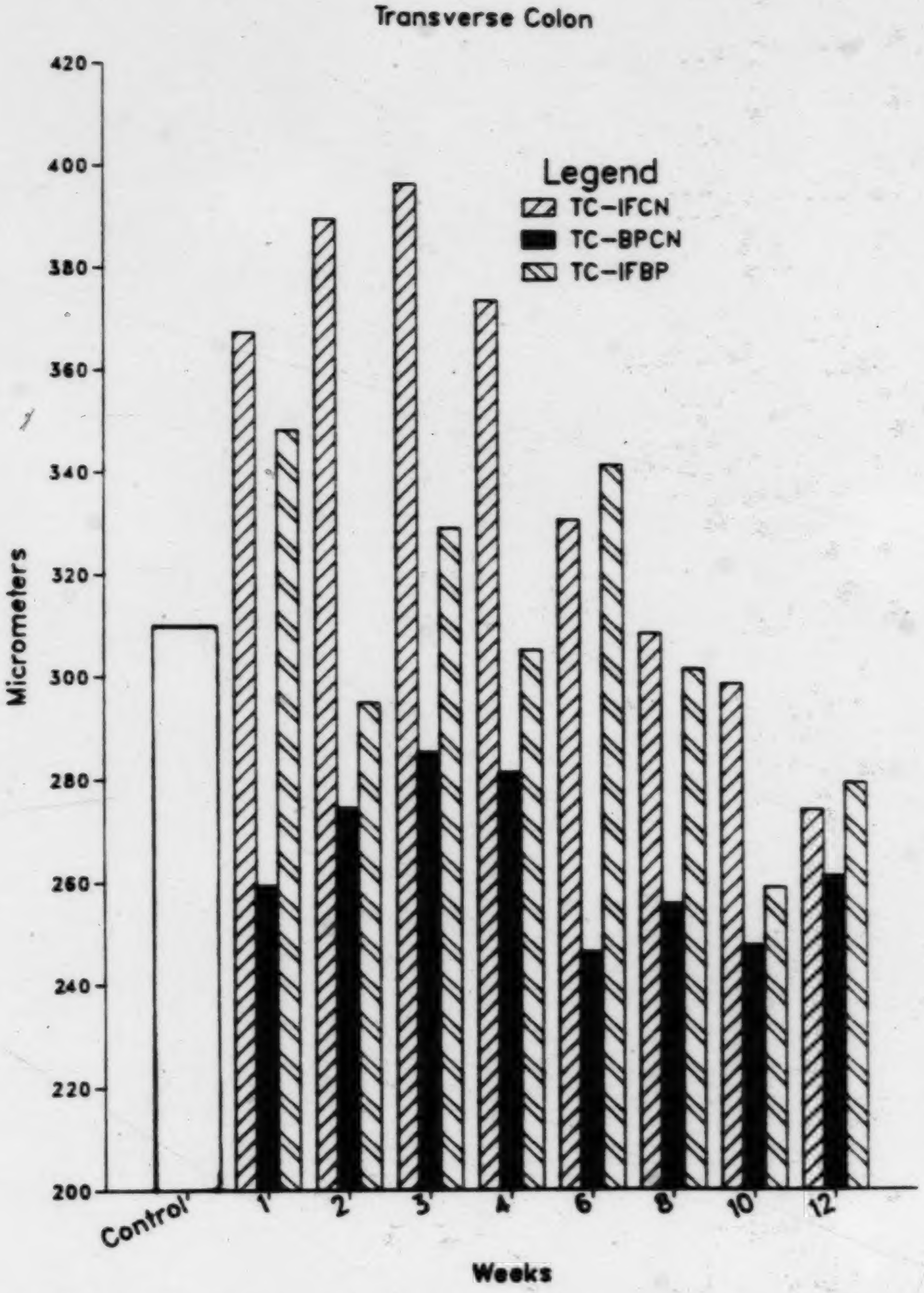

Figure IV.19b. Distance from Crypt Base to Tip of Surface Epithelium

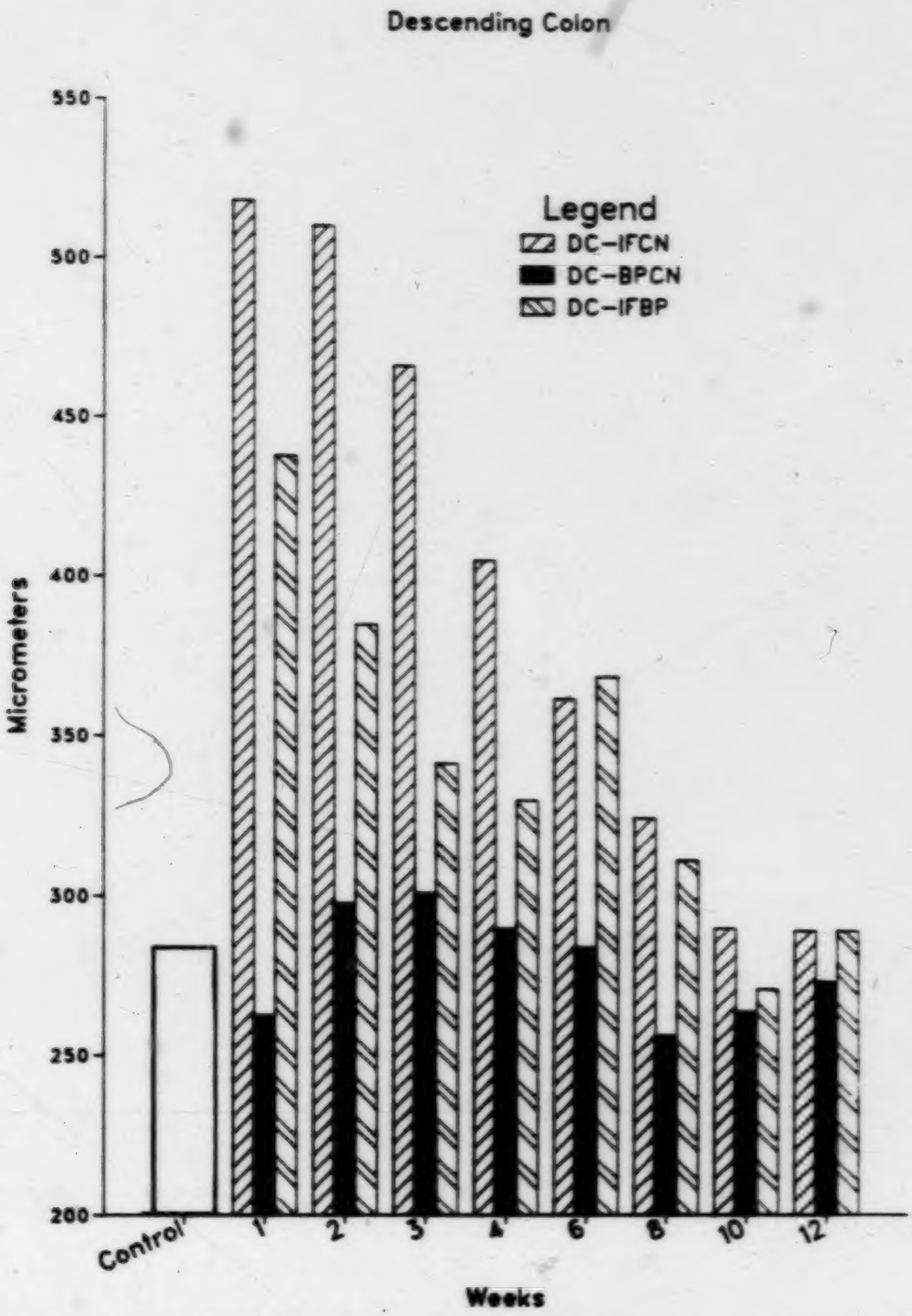

Figure IV.19c. Distance from Crypt Base to Tip of Surface Epithelium

F. Number of Mucus-containing Cells

The number of mucus-containing cells was counted in longitudinal crypts from sections stained with AB/PAS (See Figures A7.1 to A7.3). From a comparison of Figure IV.20 and Table IV.15, and Figure IV.11 and Table IV.3 several observations were made. The number of epithelial cells within the crypt column was consistent throughout the normal colon (32 cells) whereas the number of mucus cells was highest in the proximal segment (25 cells) and lowest in the descending colon (21 cells).

The response to inflammation was similar, peaking earliest in the descending colon before gradually falling off with time. At week 1 however, hyperplasia was greater for epithelial cells in general (217% of control) than for mucus-containing cells in particular (153%). By week 4 cell counts were equal for both types (137% and 135%).

Figures IV.11 and IV.20 illustrate almost identical BPCN plots. For the descending colon, both column height and mucus cell counts showed an initial week 1 decrease. However, whereas the former subsequently returned to, and was maintained at, near-normal levels; the later was delayed one week before trailing column height below the control. Similarities between curves for the ascending and transverse segments were even more striking.

IFBP plots for both parameters displayed a similar reductive trend. The main difference was the absence of the

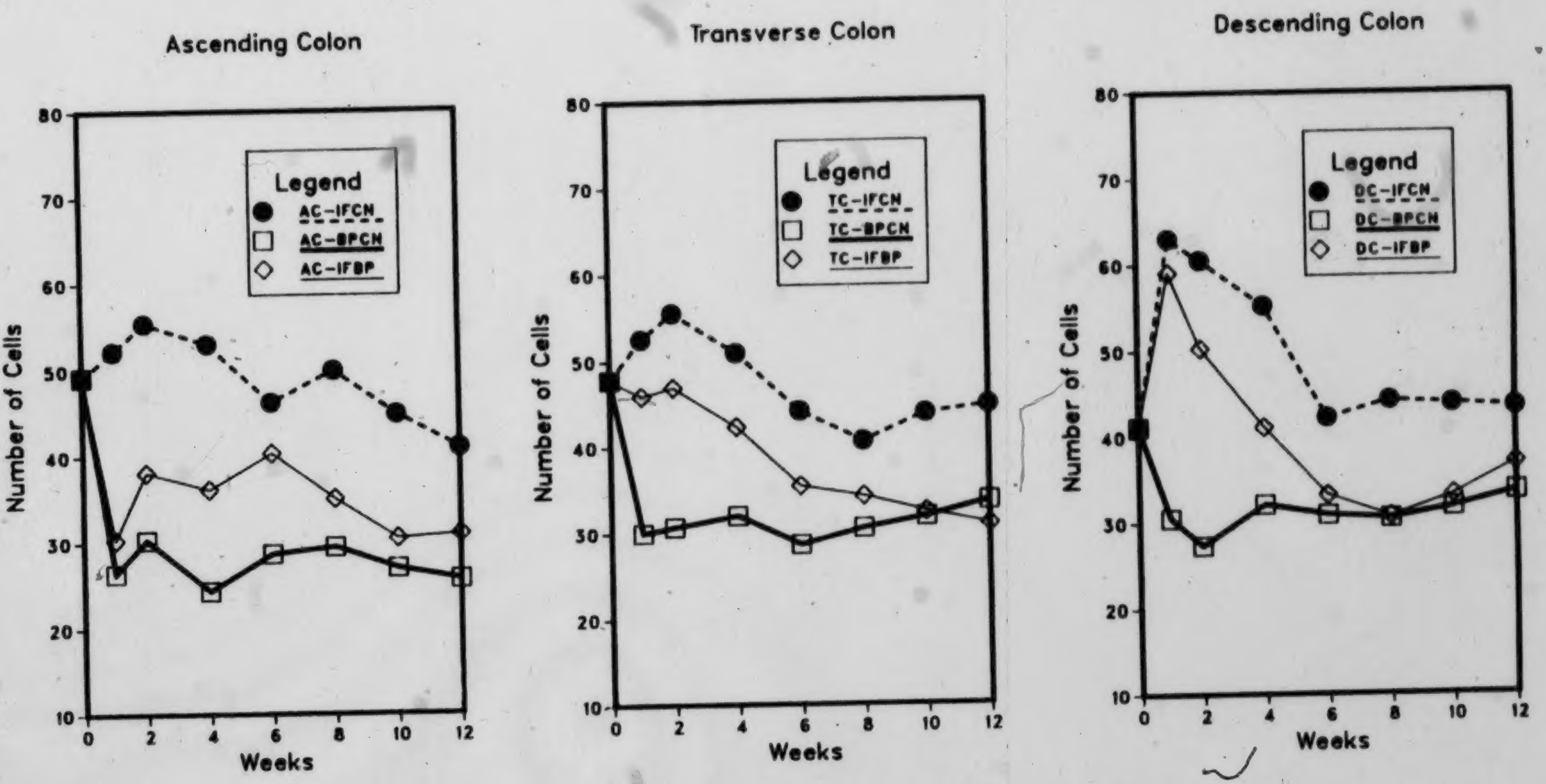

Figure IV.20. Number of Mucus-containing Cells per Longitudinal Crypt (Control: x=0,y)

Table IV.15. Number of Mucus-containing Cells per Longitudinal Crypt*

Colonic Segment	Control	One Week**			Two Weeks			Four Weeks			Six Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	49.2±7.8[1]	52.2±10.7	26.4±4.6	30.3±8.7	55.4±10.1	30.3±5.5	38.1±9.9	53.0±10.0	24.3±5.0	36.1±9.4	46.2±9.8	28.6±5.7	40.3±7.6
Transverse	47.8±6.5	52.5±9.5	30.0±5.6	45.9±13.9	55.5±10.7	30.7±6.4	46.9±12.6	50.9±11.0	32.0±5.0	42.3±10.9	44.1±10.0	28.6±5.0	35.4±9.8
Descending	41.1±2.6	63.0±7.0	30.5±2.3	59.1±15.5	60.5±6.3	27.3±3.5	50.3±12.0	55.2±7.0	32.1±3.0	41.1±13.3	42.2±6.9	30.9±2.9	33.2±8.9

Eight Weeks			Ten Weeks			Twelve Weeks		
IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
49.9±8.2	29.4±4.8	35.0±6.6	44.8±6.9	26.9±4.8	30.4±5.9	40.9±5.2	25.6±4.2	26.4±5.4
40.6±8.8	30.5±5.1	34.2±8.2	43.8±6.5	31.8±5.1	32.4±6.9	44.7±4.4	33.6±4.9	30.9±4.1
44.2±7.1	33.3±3.7	30.7±7.8	43.8±6.8	34.2±3.9	33.2±6.0	43.4±6.7	36.7±7.8	36.9±4.7

* Twice the number of mucus cells per crypt column.
** Following surgery.
[1] Mean ± SD for 5-6 animals.

Stain = AB/PAS.

inconsistent week 6 cell count.

G. Proliferative Parameters

Height of the Proliferative Zone

Photomicrographs of Figures IV.21 through IV.23 (and A7.5 to A7.6) illustrate changes in the height of the proliferative zone in response to bypass and inflammation. The height of the proliferative zone was estimated from the leading edge one hour after ^{3}HtdR label (Table IV.16, Figure IV.24) and from the highest mitotic figure along the crypt column (Table IV.17, Figure IV.25). Both variables showed that the normal colon exhibits a proximal-to-distal decrease in proliferative zone size. Expressed as the leading edge, values were 139.6, 115.8 and 102.1 micrometers for proximal, middle and distal colonic segments, respectively. Expressed as the maximum height of mitotic figures, corresponding measurements were 140.2, 112.9 and 82.0 micrometers.

In the distal colon, no significant differences were found between bypass and non-operated controls (Table IV.2). In the inflamed group by contrast, there was a 2 to 3-fold extension of the proliferative compartment at week 1. Subsequent to this sharp rise, the leading edge remained elevated above the controls while steadily falling off prior to a reversion to normal levels at week 10. Mean highest mitotic figure followed a similar course except that maximum

Figure IV.21. Autoradiographs of sections from the ascending colon: A, IFCN crypts at week 2; B, controls and C, bypassed atrophic glands. The arrow points to the leading edge 1 hour after ^{3}H-thymidine. The proliferative compartment occupies the lower two-thirds of the crypt for inflamed and normal controls but is shortened in the atrophic mucosa. MBBF, X330.

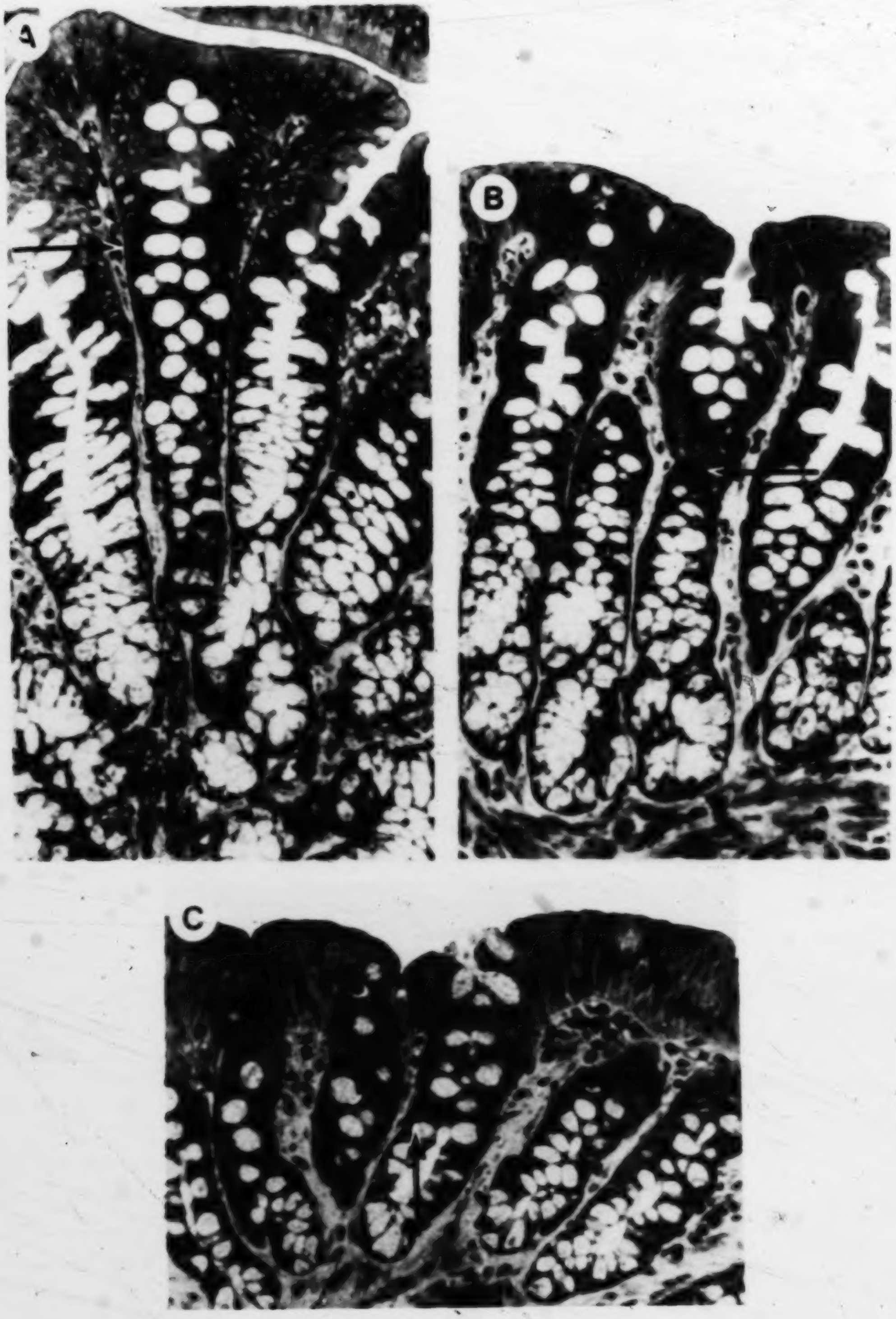
A
B
C

Figure IV.22. Autoradiographs from week 1 transverse colon for: A, inflamed controls; B, inflamed-bypass and C, bypass controls. The arrow is at the leading edge. Note the proportional extension of the leading edge seen in acetic acid-induced hyperplastic crypts which indicates no change in the relative size of the proliferative zone. MBBF, X330.

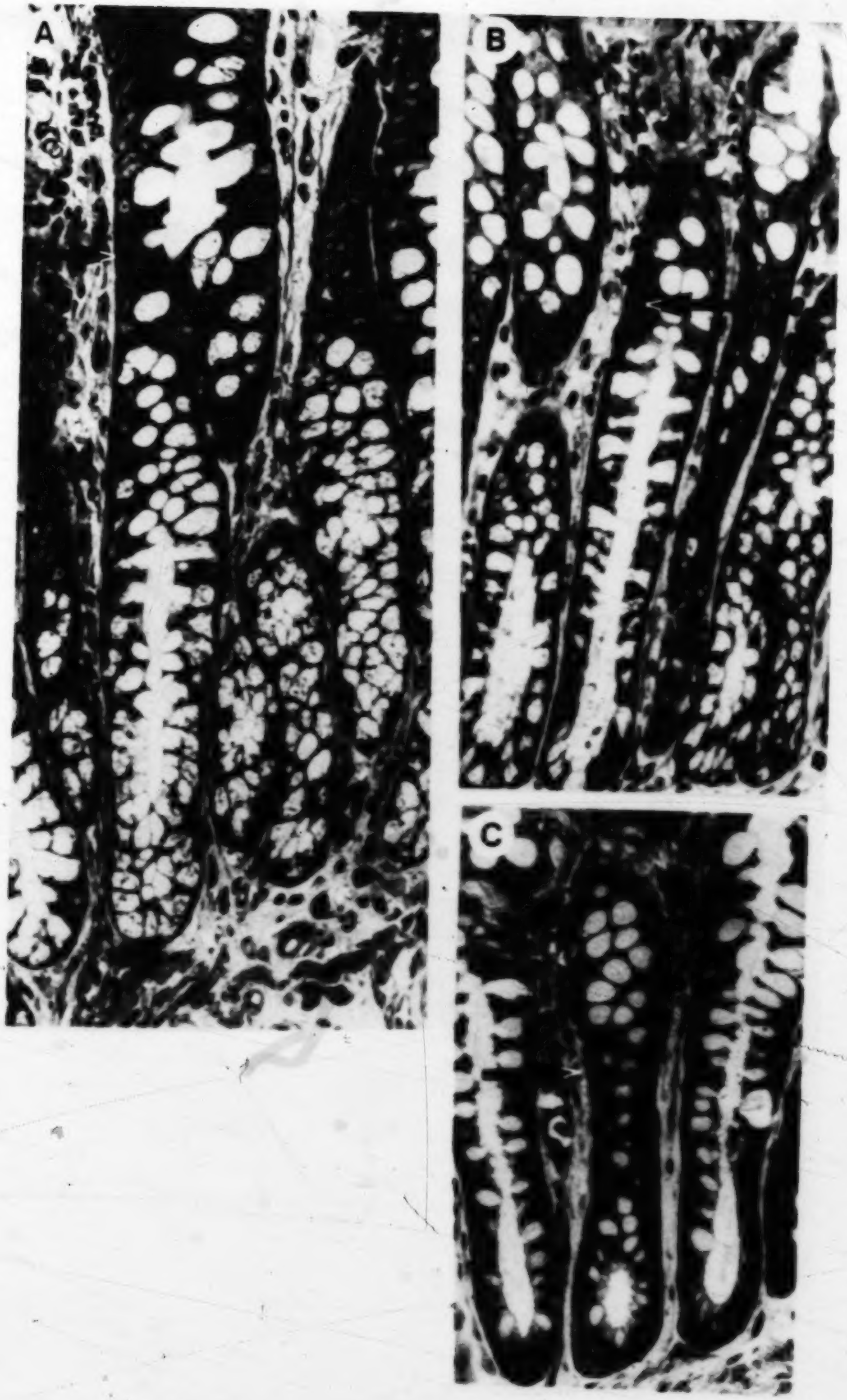
A
B
C

Figure IV.23A. Typical ulceration produced by acetic acid seen at right (distal colon). Adjacent crypts as shown below in B, are associated with hyperplastic proliferative changes. MBBF, X80.

B. Descending colonic specimen from an IFCN week 2 rat, exhibiting expansion of the progenitor area into the upper third of the crypt. This is characteristic of hyperplastic mucosa. Arrows point to 2 cells in mitosis. MBBF, X330.

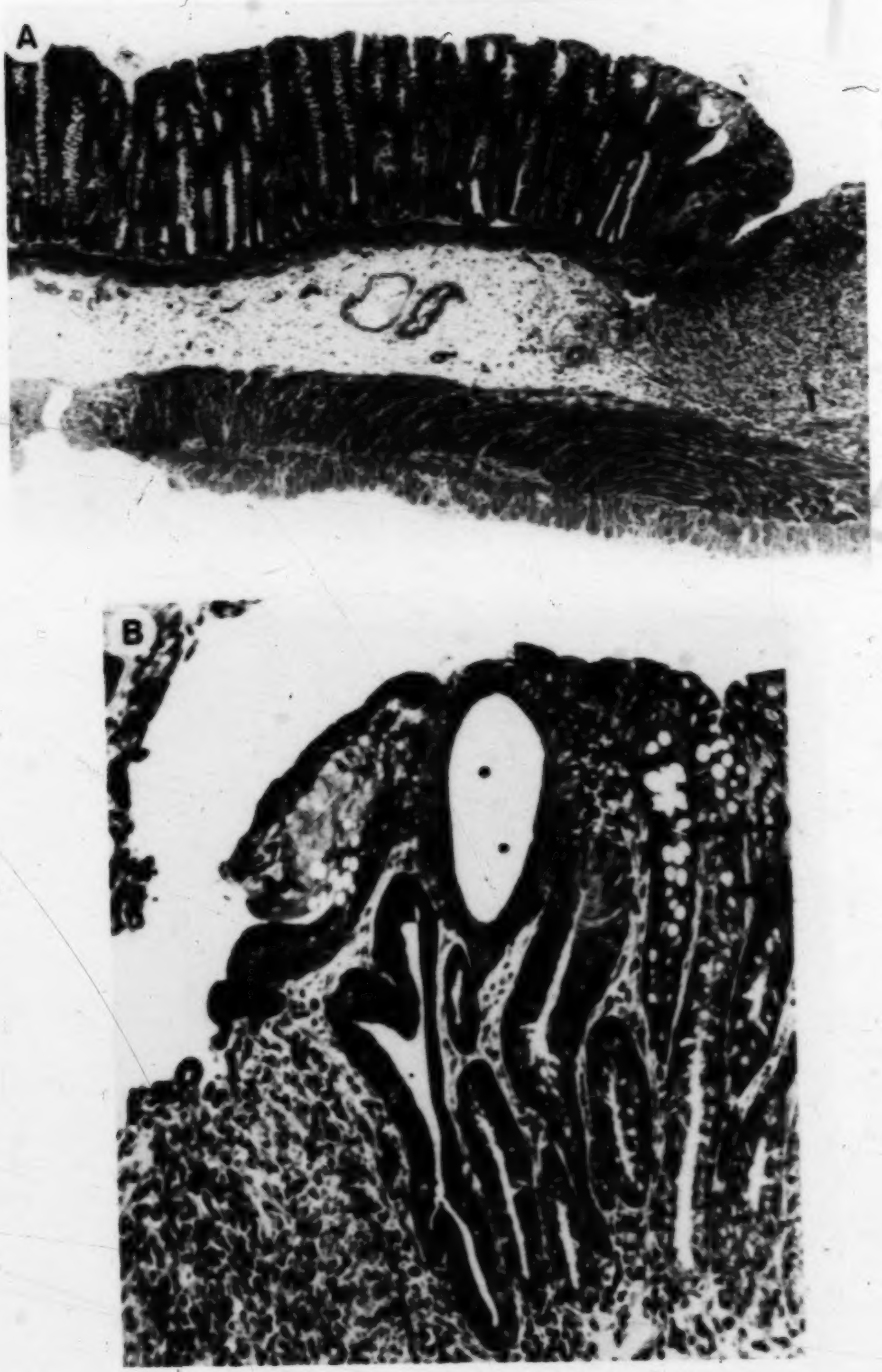
A
B

Table IV.16. Crypt Proliferative Zone Height Expressed as the Leading Edge*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	139.6±8.8[1] (3)[2]	137.4±4.5 (3)	62.4±5.2 (3)[3]	94.4±9.2 (4)	167.7±4.7 (3)	105.3±21.5 (3)[3]	70.0±1.5 (3)	166.6±13.9 (3)	85.1±5.6 (3)[3]	84.1±7.8 (3)	154.5±5.0 (4)	77.9±0.56 (2)[3]	76.5±2.8 (3)
Transverse	115.8±6.2 (3)	233.9±5.5 (3)	107.6±8.6 (3)[3]	144.0±14.4 (4)	206.1±10.96 (3)	116.3±4.3 (3)[3]	117.0±7.7 (3)	151.8±52.2 (4)	109.4±11.3 (4)[3]	108.3±27.6 (3)	211.7±14.6 (4)	103.7±10.6 (3)[3]	103.7±14.1 (4)
Descending	102.1±2.3 (3)	304.2±11.6 (3)	119.5±12.9 (3)[3]	197.2±26.0 (4)	259.6±5.7 (4)	128.8±11.7 (4)[3]	138.6±6.7 (3)	199.0±65.9 (4)	131.5±12.1 (3)[3]	134.0±3.3 (3)	218.1±19.7 (4)	117.0±7.8 (3)[3]	120.6±10.8 (3)

Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
117.9±6.6 (4)	62.7±3.2 (3)	...	164.6±5.5 (3)	77.3±10.8 (3)[3]	86.1±8.4 (4)	143.6±4.6 (3)	73.8±2.9 (3)[3]	64.4±2.5 (3)	150.6±3.2 (3)	60.2±23.1 (4)[3]	80.7±1.1 (3)
210.6±28.3 (3)	91.6±7.9 (3)	...	150.8±8.5 (3)	95.0±5.1 (3)[3]	121.2±8.9 (3)	126.8±16.5 (3)	105.7±2.1 (3)[3]	87.2±4.1 (3)	117.0±3.3 (3)	94.8±2.6 (5)[3]	106.1±3.6 (4)
179.6±29.5 (3)	112.1±5.9 (3)[3]	180.0 (1)	142.0±13.1 (3)	108.9±8.6 (3)[3]	106.7±2.0 (3)	124.4±3.2 (2)	95.4±4.0 (3)[3]	95.9±2.6 (3)	117.5±1.0 (3)	95.2±23.8 (5)[3]	114.5±4.4 (4)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	3.18	<0.001	<0.05

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* From ^{3}H-Thymidine Lable.
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.
[4] No significant differences between the 3 groups.
[5] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.
[6] IF/BP and IF/CN are significantly different from each other but not from BP/CN.

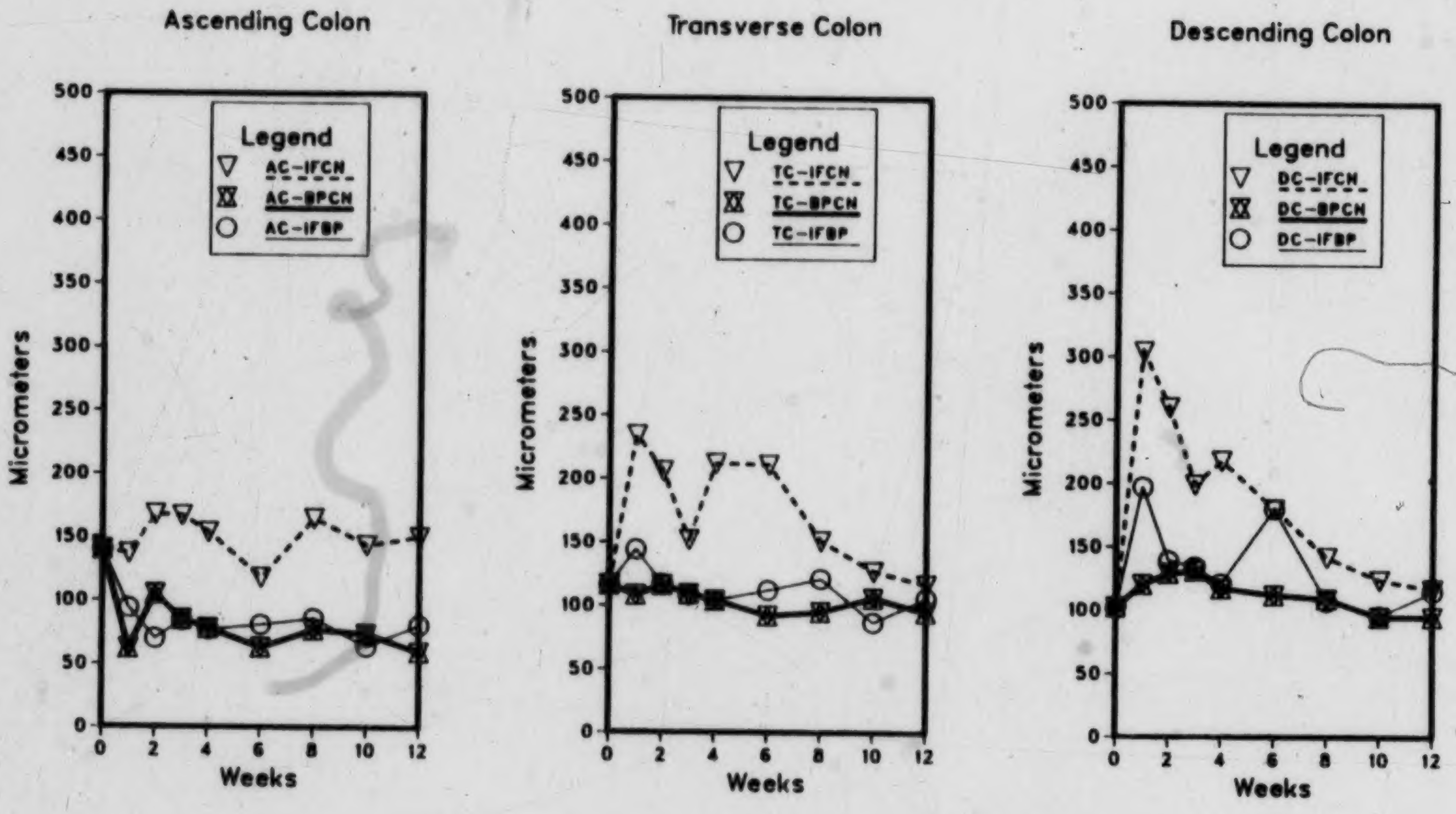

Figure IV.24. Height of Proliferative Zone from Tritiated Thymidine Label (Control: x=0,y)

Table IV.17. Maximum Height of Mitotic Figures Along Crypt Column*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	140.2±6.8^{1} (12)^{2}	125.6±4.5 (6)	49.7±6.4 (6)^{3}	94.9±15.3 (6)	159.3±6.7 (6)	70.2±11.6 (6)^{3}	57.8±6.1 (6)	145.3±8.3 (7)	62.2±6.0 (6)^{3}	92.2±9.0 (5)	148.2±7.6 (7)	69.8±5.66 (6)^{3}	61.4±6.3 (6)
Transverse	112.9±6.9 (12)	165.3±20.3 (6)	74.7±10.2 (6)^{3}	123.7±14.9 (6)	169.7±14.7 (6)	80.4±17.0 (6)^{3}	105.5±6.8	161.7±14.8 (8)	70.9±7.5 (6)^{3}	106.2±9.9 (6)	138.5±16.3 (8)	86.8±5.4 (6)^{3}	106.3±16.9 (6)
Descending	82.0±3.4 (12)	164.7±10.7 (6)	77.7±9.4 (6)^{4}	164.7±28.5 (6)	161.6±7.6 (6)	94.9±4.9 (6)^{3}	123.1±13.4 (7)	178.3±10.7 (7)	77.2±8.2 (6)^{3}	100.7±6.6	148.1±17.5 (6)	70.1±5.5 (6)^{3}	88.2±11.6 (6)

Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
119.3±6.1 (6)	53.5±3.7 (6)^{3}	107.6±31.2 (5)	147.7±7.5 (6)	66.8±6.2 (6)^{3}	65.4±7.4 (5)	151.4±4.2 (7)	49.7±6.5 (6)^{3}	58.1±6.7 (6)	160.2±5.1 (6)	68.9±4.5 (6)^{3}	75.1±9.2 (6)
166.3±15.3 (6)	66.8±9.8 (6)^{3}	129.8±29.6 (5)	126.9±9.0 (6)	70.7±6.2 (6)^{3}	93.4±10.8 (5)	120.2±5.4 (7)	74.3±4.3 (6)^{3}	55.0±7.5 (6)	132.6±5.4 (9)	76.5±4.1 (6)^{3}	76.6±6.1 (6)
127.8±15.0 (6)	83.2±7.1 (6)^{4}	133.3±30.0 (5)	109.3±15.4 (6)	69.8±2.8 (5)^{3}	80.8±12.5 (4)	87.0±3.2 (6)	74.4±6.5 (6)^{3}	78.1±4.7 (7)	85.6±4.7 (8)	75.5±3.5 (5)^{3}	93.6±8.6 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	1.99	<0.01	<0.05

* Micrometers.

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (AC etc.) but not from each other.

[5] No significant differences between the 3 groups.

[6] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

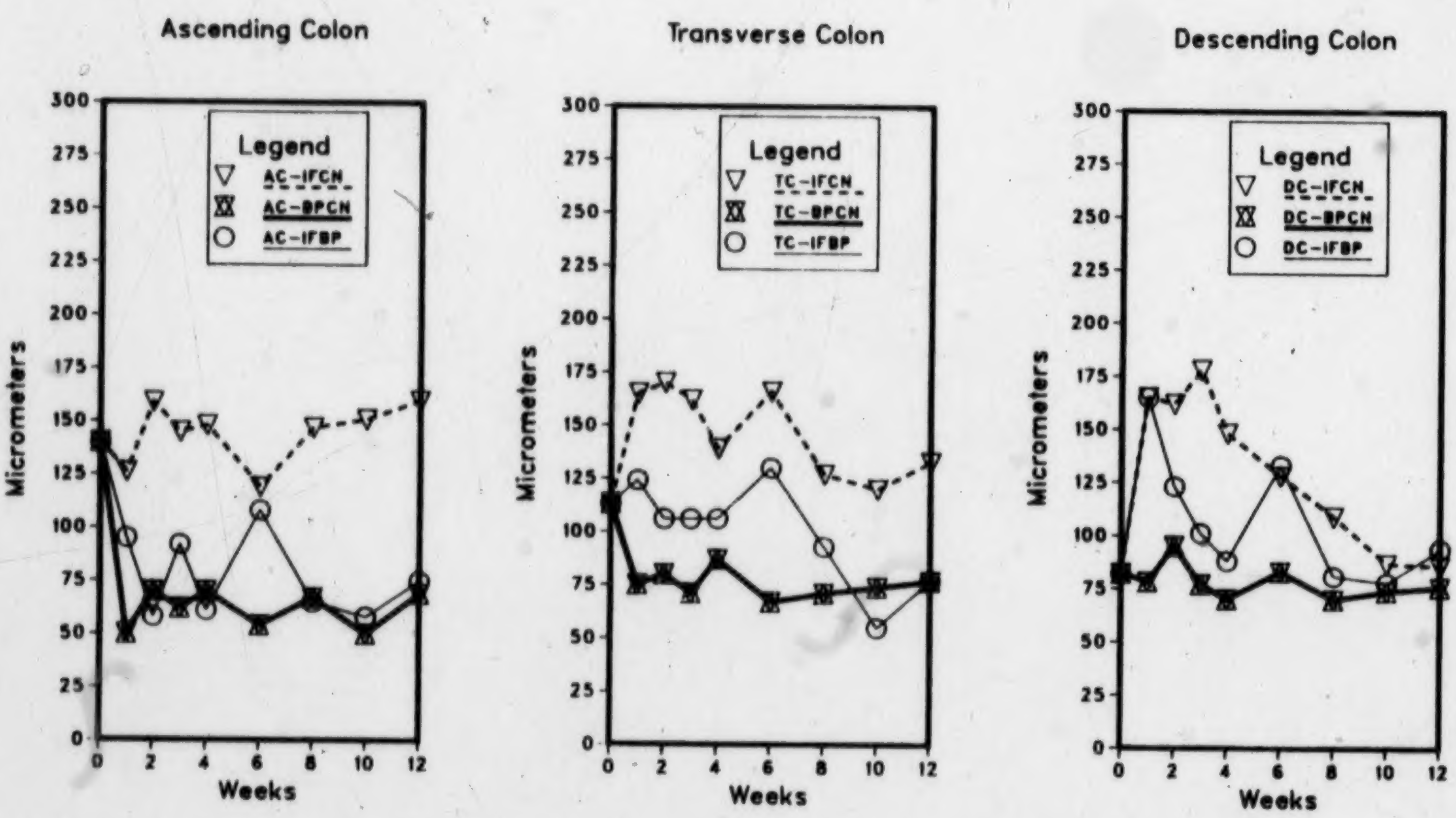

Figure IV.25. Height of Proliferative Zone from Highest Mitotic Figure (Control: x=0,y)

expansion occurred at week 3.

Bypassing the inflamed colon resulted in smaller increments than after treatment with acetic acid only. This was evident as early as the first week for the leading edge (a little later for mitotic figures); the progenitor area of IFBP crypts being 54% shorter than IFCN and 39% taller than BPCN. The difference from inflamed controls achieved significance from week 1 to week 4. With the exclusion of the elevated IFBP values observed at week 6, fecal diversion resulted in a near-normal recovery within 4 weeks. This probably indicates the counteractive effect of bowel rest on the exaggerated cell turnover associated with the inflammatory process.

In the transverse colon of bypass controls, values were substantially below normal, particularly when expressed as mean highest mitotic figure (Table IV.2). Crypts with shortened proliferative zones became a constant feature from week 1 to week 12. In the inflamed controls, there was a maximum extension of the leading edge of about twice normal controls after 1 week with a concomitant rise of similar magnitude of the leading mitotic figure. Increased heights persisted until week 6 but then normalized at week 8. Again, experimental bypass resulted in recovery from inflamed heights; 3 weeks following surgery.

The greatest degree of proliferative zone shortening was seen in the defunctioned proximal colon. The variation from normal (and inflamed controls) was significant for all time intervals for the bypass controls but was delayed one week in the IFBP group. In the corresponding segment of the inflamed controls, there was a temporary expansion (insignificant) at week 2 with a subsequent normalization at week 4; probably indicating a delayed but transient inflammatory response.

The proliferative zone height response to bypass and/or inflammation generally mirrored the results of the average height of mitotic figures from the crypt base (Table IV.18, Figure IV.26) and was consistent with those for other variables, including total crypt cell population, column height and measured crypt height. It was not surprising, therefore, that there was a highly significant and close correlation (r=0.83 to 0.85) between these parameters and the leading edge (Table IV.2).

Mitotic Count and Index

The marked hypoplasia and mucosal atrophy observed in the excluded ascending colon of the operated groups were further substantiated by the data for mitotic counts (Table IV.19, Figure IV.27) and mitotic index (Table IV.20). With respect to the first proliferative parameter, both BPCN and IFBP values showed a consistent pattern of reduction. The mean number of mitotic figures per crypt was reduced to respectively, 21% and 90% of controls for the first weekly interval. Thereafter, values for both groups were usually similar and significantly lower than non-operated and

Table IV.18. Average Height of Mitotic Figures from Crypt Base (Micrometers)

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	136.1±7.6[1] (12)[2]	121.6±4.5 (6)	49.7±6.4 (6)[3]	92.1±13.5 (6)	157.4±6.7 (6)	70.2±11.6 (6)[3]	57.8±6.1 (6)	139.6±8.2 (7)	62.2±6.0 (6)[3]	91.4±1.8 (5)	144.7±7.8 (7)	69.8±5.6 (6)[3]	61.2±6.1 (6)
Transverse	106.9±6.5 (12)	157.1±18.1 (6)	68.8±8.1 (6)[4]	122.5±15.2 (6)	159.2±13.3 (6)	79.2±17.0 (6)[3]	103.2±6.9 (6)	152.7±15.4 (8)	69.9±7.6 (6)[3]	104.8±9.7 (6)	130.5±16.0 (8)	83.8±4.5 (6)[3]	106.2±16.9 (6)
Descending	76.4±2.4 (12)	139.2±8.6 (6)	70.7±8.2 (6)[4]	145.5±20.0 (6)	135.2±5.9 (6)	90.0±4.7 (6)[3]	114.8±11.8 (7)	149.6±9.0 (7)	73.6±8.3 (6)[3]	94.5±5.9 (6)	137.2±16.6 (6)	73.7±5.1 (6)[3]	87.5±12.0 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	116.9±6.1 (6)	53.5±3.6 (6)[3]	102.4±28.4 (6)	146.7±7.2 (6)	66.8±7.0 (6)[3]	65.1±10.8 (5)	150.7±4.4 (7)	49.7±6.5 (5)[3]	58.1±7.5 (6)	157.7±4.8 (6)	68.1±4.4 (6)[3]	75.1±5.9 (6)
	154.5±13.7 (6)	64.8±9.4 (6)[4]	123.1±26.6 (5)	114.2±8.0 (6)	67.4±6.1 (6)[3]	93.4±10.8 (5)	111.7±6.4 (7)	73.2±4.4 (6)[6]	54.0±7.5 (5)	122.7±7.0 (9)	76.5±4.1 (6)[5]	75.5±5.9 (6)
	144.6±12.3 (6)	80.4±5.9 (6)[5]	112.8±23.3 (5)	99.2±13.5 (6)	68.2±2.6 (5)[5]	78.3±11.4 (4)	83.2±3.2 (6)	73.1±6.6 (6)[5]	77.6±4.6 (7)	80.8±5.4 (8)	74.7±3.6 (5)[5]	87.6±8.0 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F		P
Segment x Group x Week	2.08	<0.003	<0.05

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[4] IF/BP (AC etc.) and IF/CN (AC etc.) are significantly different from BP/CN (etc.) but not from each other.

[5] No significant differences between the 3 groups.

[6] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

[7] IF/BP and IF/CN are significantly different from each other but not from BP/CN.

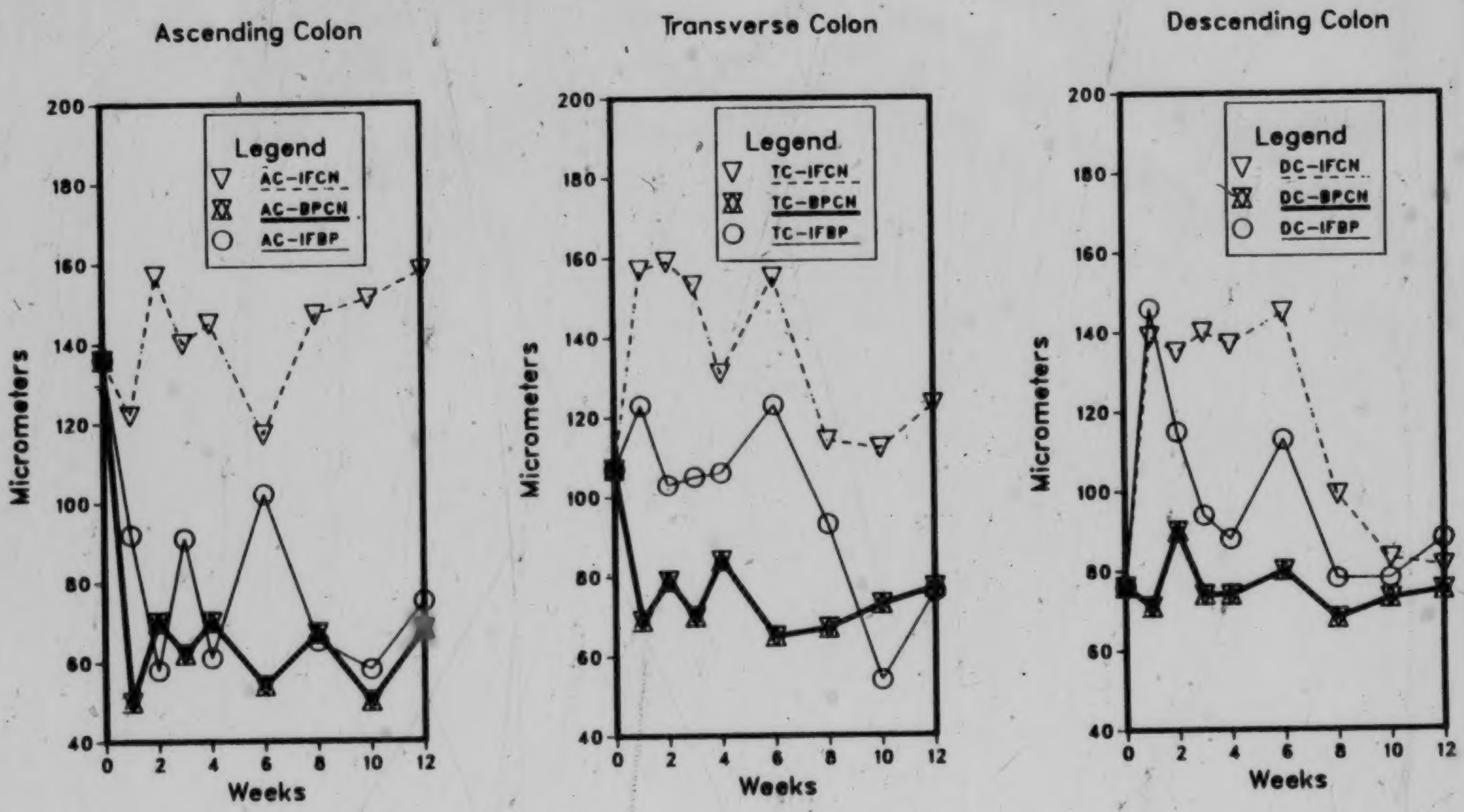

Figure IV.28. Average Height of Mitotic Figures from Crypt Base (Control: x=0,y)

Table IV.19. Number of Mitotic Figures Seen in Longitudinally Cut Crypts

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	0.565±0.05[1] (12)[2]	0.649±0.02 (6)	0.117±0.01 (6)[3]	0.511±0.11 (6)	0.509±0.05 (6)	0.167±0.05 (6)[3]	0.142±0.04 (6)	0.643±0.06 (7)	0.129±0.03 (6)[3]	0.202±0.07 (6)	0.571±0.04 (7)	0.060±0.03 (5)[3]	0.164±0.04 (6)
Transverse	0.738±0.13 (12)	0.740±0.06 (6)	0.500±0.20 (6)[3]	0.398±0.03 (6)	0.833±0.08 (6)	0.283±0.07 (6)[3]	0.336±0.07 (6)	0.687±0.11 (8)	0.238±0.03 (6)[3]	0.389±0.02 (6)	0.745±0.10 (8)	0.347±0.07 (6)[6]	0.045±0.25 (6)
Descending	0.585±0.10 (12)	1.372±0.07 (6)	0.702±0.24 (6)[3]	0.794±0.25 (6)	1.364±0.16 (6)	0.596±0.12 (6)[3]	0.648±0.20 (7)	1.477±0.15 (7)	0.476±0.07 (6)[3]	0.606±0.13 (6)	0.883±0.16 (6)	0.411±0.14 (6)[3]	0.311±0.04 (6)

Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
0.614±0.06 (6)	0.146±0.03 (6)[3]	0.588±0.22 (5)	0.567±0.08 (6)	0.198±0.04 (6)[3]	0.231±0.10 (6)	0.389±0.03 (7)	0.156±0.04 (6)[3]	0.183±0.02 (6)	0.372±0.02 (6)	0.189±0.10 (6)[3]	0.200±0.04 (6)
0.861±0.11 (6)	0.42±0.08 (6)[3]	0.553±0.10 (5)	0.775±0.19 (6)	0.492±0.10 (6)[3]	0.207±0.03 (5)	0.899±0.09 (7)	0.375±0.05 (6)[3]	0.375±0.07 (6)	0.872±0.11 (9)	0.446±0.04 (6)[6]	0.392±0.09 (6)
0.661±0.15 (6)	0.421±0.20 (6)[3]	0.737±0.11 (5)	0.508±0.11 (6)	0.477±0.07 (5)[5]	0.131±0.06 (6)	0.622±0.04 (6)	0.421±0.04 (6)[3]	0.326±0.06 (7)	0.642±0.06 (8)	0.438±0.03 (5)[3]	0.418±0.08 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.12	<0.01	<0.05

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[3] IF/BP (AC, TC or DC) and BP/CN (AC, TC or DC) are significantly different from IF/CN (AC, TC or DC) but not from each other.

[4] No significant differences between the 3 groups.

[5] IF/BP is not significantly different from either BP/CN or IF/CN; the last two being significantly different from each other.

[6] IF/BP and IF/CN are significantly different from each other but not from BP/CN.

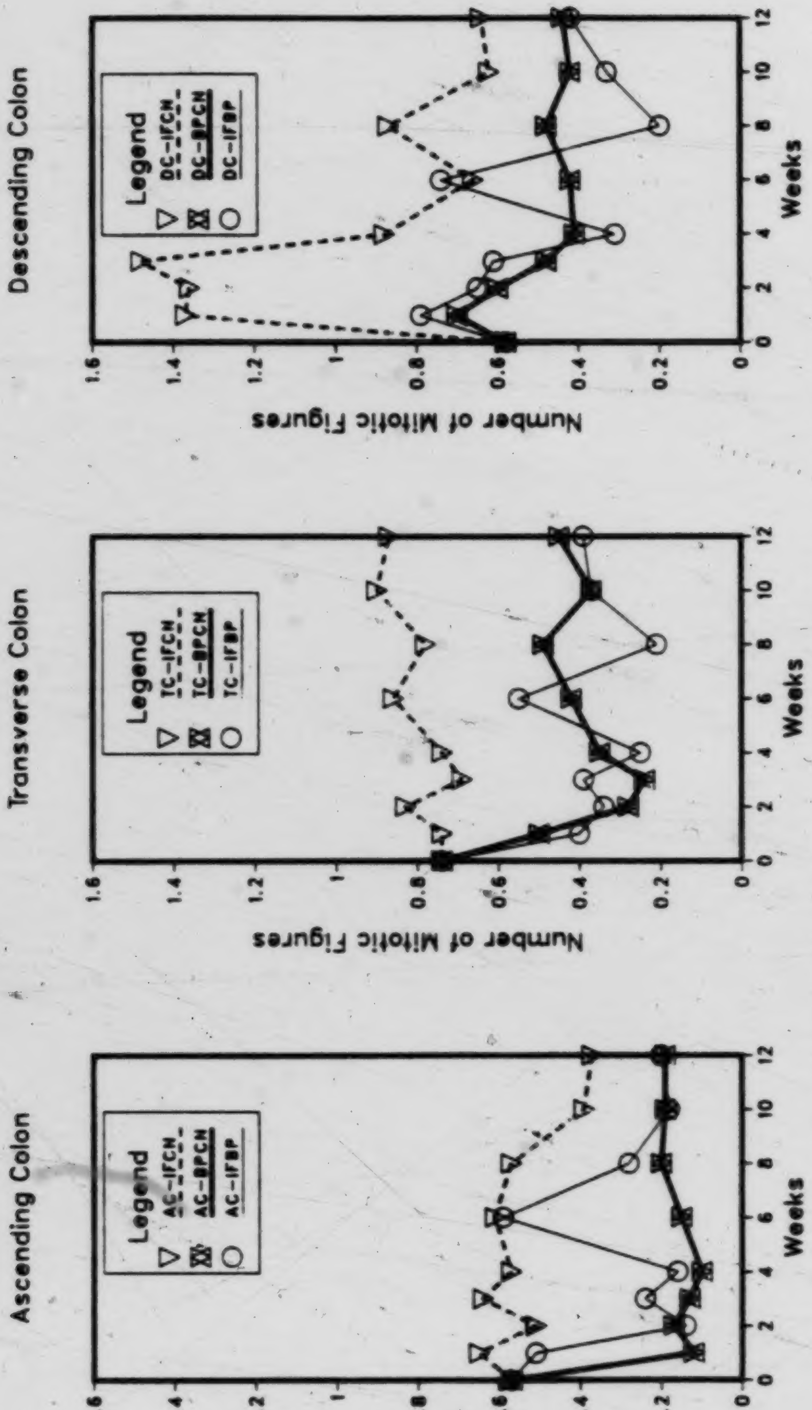

Figure IV.27. Number of Mitotic Figures Seen in Longitudinally Cut Crypts (Control: x=0,y)

Table IV.20. Crypt Mitotic Index*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	1.821±0.200[1] (12)	2.085±0.097 (6)	0.635±0.099 (6)	1.776±0.283 (6)	1.598±0.190 (6)	0.639±0.177 (6)	0.716±0.193 (6)	1.907±0.157 (7)	0.529±0.139 (6)	0.844±0.284 (6)	1.841±0.172 (7)	0.328±0.159 (5)	0.771±0.187 (6)
Transverse	2.364±0.460 (12)	1.872±0.214 (6)	1.937±0.767 (6)	1.129±0.186 (6)	2.051±0.190 (6)	1.159±0.335 (6)	1.188±0.243 (6)	1.665±0.283 (8)	0.910±0.097 (6)	1.270±0.081 (6)	1.769±0.232 (8)	1.338±0.273 (6)	0.856±0.151 (6)
Descending	1.823±0.299 (12)	1.946±0.098 (6)	2.354±0.774 (6)	1.373±0.310 (6)	2.243±0.312 (6)	1.870±0.401 (6)	1.491±0.465 (7)	3.055±0.349 (7)	1.485±0.221 (6)	1.593±0.368 (6)	1.985±0.340 (6)	1.349±0.443 (6)	0.861±0.149 (6)

Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
2.201±0.214 (6)	0.816±0.117 (6)	2.071±0.471 (5)	1.724±0.228 (6)	0.925±0.186 (6)	1.095±0.444 (6)	1.224±0.108 (7)	0.835±0.181 (6)	0.912±0.084 (6)	1.095±0.081 (6)	0.888±0.399 (6)	0.935±0.224 (6)
2.552±0.313 (6)	1.709±0.320 (6)	1.582±0.147 (5)	2.364±0.511 (6)	1.829±0.332 (6)	0.709±0.125 (5)	2.854±0.300 (7)	1.455±0.171 (6)	1.448±0.310 (6)	2.710±0.351 (9)	1.725±0.172 (6)	1.513±0.376 (6)
1.675±0.258 (6)	1.353±0.308 (6)	1.840±0.141 (5)	1.394±0.211 (6)	1.593±0.202 (5)	0.398±0.202 (6)	1.954±0.132 (6)	1.424±0.115 (6)	1.070±0.18[1] (7)	1.908±0.176 (8)	1.462±0.086 (5)	1.244±0.230 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.40	<0.001	<0.05 [3]NC

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* Expressed as the number of mitotic figures in a longitudinal gland divided by the number of cells per crypt column X100.
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.
For Scheffe's Multiple Comparisons:
[3] NC = Not calculated for Segment x Group x Week (See Table IV.2).

inflamed controls. Alternatively, in the inflamed controls mitotic counts had a tendency to fluctuate around the control and showed a slight, but insignificant, drop at weeks 10 to 12.

The pattern of change in the transverse colon, on the other hand, was less clear. As Table IV.2 shows, IFCN did not deviate appreciably from the unoperated rats. However, despite a fair amount of fluctuation, BPCN and IFBP exhibited a decreasing tendency that reached statistical significance for most of the weekly intervals.

In the descending colon, there was an initial week 1 increase in all three groups; the most marked being IFCN, followed by IFBP and BPCN, respectively. The IFCN counts remained in excess of twice the control; peaking at week 3 but afterwards declined steadily before normalizing at week 8. Subsequent to the initial BPCN increase, values returned to normal prior to falling insignificantly below that level. With respect to IFBP rats, following the week 1 increase that was 58% of inflamed values, mitotic counts returned to normal by week 3 and subsequently, generally fell insignificantly below the control.

The mitotic index response pattern to bypass (BPCN), inflammation (IFCN) or both (IFBP) was almost equal to that of mitotic counts, particularly in the ascending and transverse colon. As before, differences in these two segments were generally statistically significant (Table IV.2). In the case of the distal colon, the similarity was slightly less striking. For inflamed controls, both variables peaked simultaneously at week 3. However, a substantial increase over control values occurred during the third week for mitotic index and during the first for mitotic count. The BPCN values again, exhibited the transient increase at week 1 before patterning IFBP and falling insignificantly below the control.

Relative Size of the Proliferative Zone

Autoradiographic data showed no significant variation in the relative size of the proliferative zone between the four groups in all colonic segments (Table IV.21). When estimated from mitotic height (Table IV.22, Figure IV.28), however, this parameter showed significant differences between the proximal and middle segments of bypass controls on the one hand and those of inflamed and nonoperated controls on the other (Table IV.2). A similar situation existed for the IFBP rats but transverse colonic crypts in this group did not deviate significantly from normal.

As Figure IV.28 shows, while proximal colonic values for the surgically treated groups decreased slightly from week 1 to week 12, they were largely similar to normal in the inflamed controls. In the transverse colon, bypass (BPCN) promoted an adaptive response that was similar in magnitude and temporal course to that of the proximal region. Additionally, inflammation (IFCN) appeared to stimulate

Table IV.21. Relative Size of Proliferative Zone from Tritiated Thymidine Label*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	63.7±2.7[1] (3)[2]	59.9±1.4 (3)	55.7±9.0 (3)	51.1±6.2 (4)	62.9±0.9 (3)	57.1±4.7 (3)	49.4±2.6 (3)	62.5±0.7 (3)	59.9±8.6 (3)	45.4±2.3 (3)	59.3±2.2 (4)	60.0±2.4 (2)	53.9±2.1 (3)
Transverse	43.2±1.9 (3)	61.1±2.6 (3)	48.4±3.6 (3)	47.7±2.3 (4)	62.8±3.1 (3)	51.7±3.0 (3)	47.0±1.9 (4)	42.9±14.0 (4)	43.3±4.4 (3)	37.2±10.4 (3)	62.1±4.3 (4)	42.3±2.4 (3)	40.7±3.7 (4)
Descending	41.6±1.6 (3)	61.1±2.0 (3)	53.8±3.7 (3)	51.1±1.7 (3)	56.8±2.6 (4)	52.6±6.5 (3)	43.2±5.2 (3)	45.3±14.9 (4)	52.7±3.9 (3)	46.2±0.1 (3)	54.9±1.8 (4)	45.3±3.0 (3)	38.2±3.2 (3)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	62.6±2.4 (4)	51.5±4.6 (3)	---	69.7±3.3 (3)	57.8±5.4 (3)	56.7±2.8 (4)	61.0±4.2 (3)	55.5±2.9 (3)	47.8±0.2 (3)	61.5±3.3 (3)	46.2±16.5 (4)	46.5±2.0 (3)
	64.2±2.2 (3)	45.1±2.0 (3)	---	55.4±1.3 (3)	42.7±2.6 (3)	45.8±2.4 (3)	46.3±5.4 (3)	47.8±0.4 (3)	43.2±2.2 (3)	42.1±1.3 (3)	43.2±1.2 (5)	42.5±1.6 (4)
	53.6±6.2 (3)	43.8±0.2 (3)	55.6 (1)	50.4±1.9 (3)	49.1±5.8 (3)	42.7±0.6 (3)	50.4±2.1 (2)	43.6±1.6 (3)	41.0±1.5 (3)	48.4±3.5	42.2±10.8 (5)	44.5±1.7 (4)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	0.69	=0.8304 [3]NS	<0.05

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* Expressed as the percentage of crypt column height in micrometers.
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

[3] NS = Not Significant.
For Scheffe's Multiple Comparisons:
No significant differences between the 3 groups.

Table IV.22. Relative Size of Proliferative Zone Estimated from the Highest Mitotic Figure*

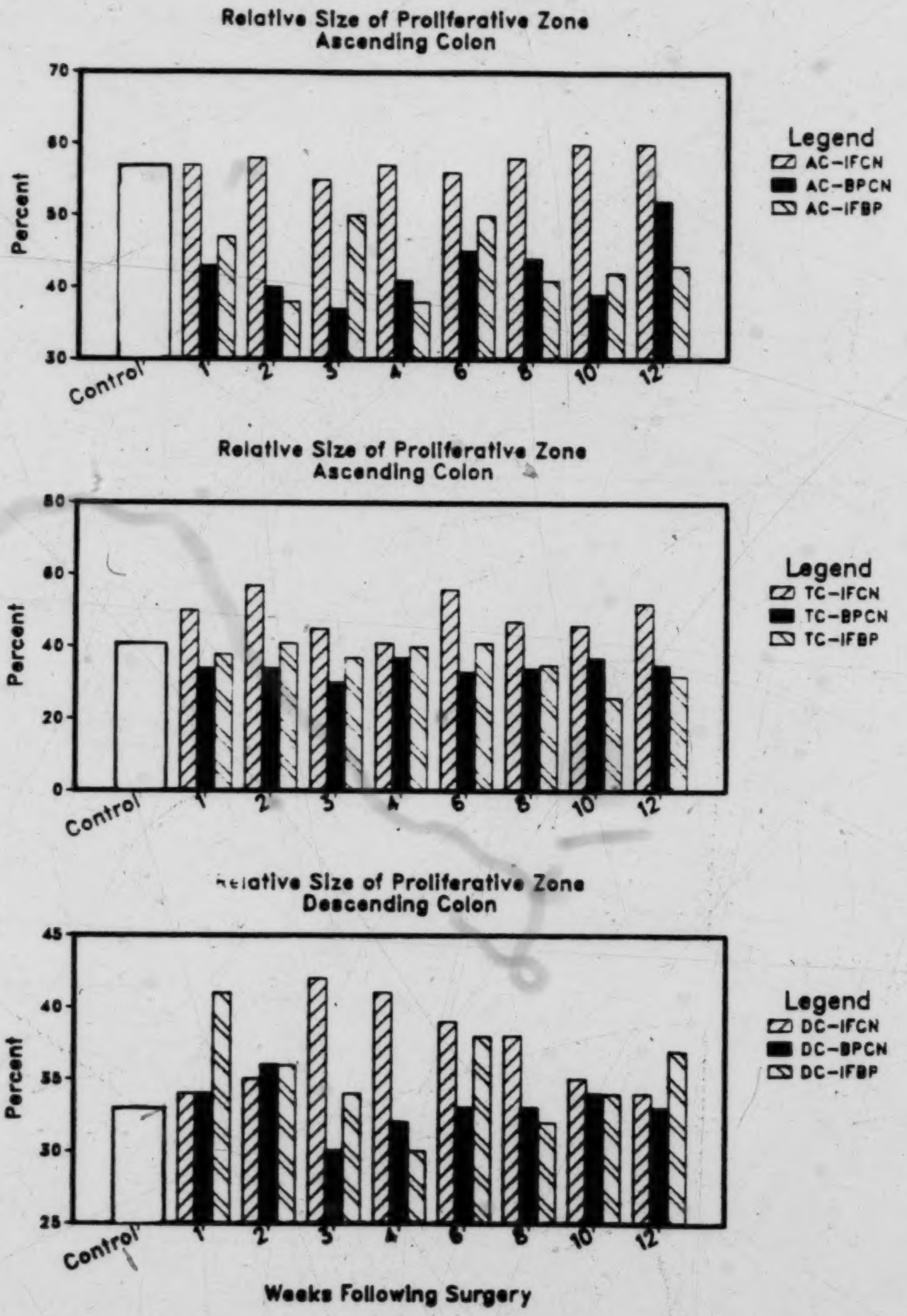
Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	56.9±1.7[1] (12)[2]	57.0±2.4 (6)	43.6±6.0 (6)	46.7±2.4 (6)	57.8±1.8 (6)	40.5±4.6 (6)	38.2±3.5 (6)	55.2±1.6 (7)	40.0±3.8 (6)	49.8±3.7 (5)	57.2±3.0 (7)	45.3±3.6 (3)	37.6±2.9 (6)
Transverse	41.4±2.4 (12)	50.4±3.5 (6)	33.9±3.9 (6)	38.2±2.3 (6)	57.0±12.1 (6)	34.5±6.8 (6)	40.9±2.7 (6)	45.2±2.9 (8)	29.8±2.2 (6)	36.9±3.1 (6)	40.8±4.1 (8)	36.8±1.6 (6)	40.1±5.4 (6)
Descending	33.4±1.5 (12)	33.9±2.5 (6)	34.4±3.1 (6)	40.7±4.3 (6)	35.2±2.3 (6)	36.1±2.4 (6)	35.9±3.2 (7)	41.6±1.6 (7)	29.7±2.7 (6)	34.0±2.3 (6)	40.9±3.2 (6)	31.8±2.2 (6)	30.4±3.6 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		56.2±4.2 (6)	45.0±2.4 (6)	49.8±3.9 (5)	57.8±2.3 (6)	43.8±3.2 (6)	41.1±4.6 (5)	60.5±1.3 (7)	39.3±4.0 (5)	41.7±3.6 (6)	60.4±1.9 (6)	52.0±3.9 (6)	43.1±5.7 (6)
		55.9±1.6 (6)	32.7±4.8 (6)	40.8±4.5 (5)	46.9±2.1 (6)	33.6±2.7 (6)	35.3±3.1 (5)	46.3±2.5 (7)	37.1±3.27 (6)	25.6±3.3 (6)	52.0±2.2 (6)	34.9±2.4 (6)	31.9±2.8 (6)
		39.3±2.7 (6)	32.9±2.8 (6)	38.3±3.5 (5)	37.7±3.9 (6)	33.2±2.5 (5)	32.2±6.0 (5)	34.8±1.3 (6)	33.6±3.48 (6)	34.4±1.8 (7)	34.4±2.0 (8)	33.4±2.7 (5)	37.3±3.6 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	1.64	<0.05	<0.05

* Expressed as the percentage of crypt column height in micrometers.
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
No significant differences between the 3 groups.



Relative Size of Proliferative Zone
Ascending Colon

Relative Size of Proliferative Zone
Ascending Colon

Relative Size of Proliferative Zone
Descending Colon

Figure IV.28. Relative Size of Proliferative Zone

irregular increases at weeks 1 to 2, 6 and 12. In marked contrast, proliferative zone size of IFBP crypts underwent significant alterations and were 32% to 39% shorter than IFCN within 7 to 14 days. In the distal colon, the relative size of the progenitor area showed few and inconsistent differences between all four groups as did the autoradiographic results.

H. Mucin Histochemistry

Descending Colon

KOH/AB1.0/PAPS

Control slides stained with this histochemical procedure indicated that crypts of the descending colon are predominantly sulphomucin-secreting, staining aqua in colour. This did not change throughout the 12 week period in the BPCN rats. Animals exposed to the inflammatory reaction (IFCN) demonstrated the typical shift towards sialomucin production (red/fuschia) which was localized in the base, or scattered throughout the crypts. The staining characteristics of this region had returned to normal within 12 weeks. The IFBP group also showed this abnormal mucin pattern (red/fuschia) but had reduced sialomucin staining compared to inflamed controls from week 1. The mucin profile seen at 1 week in IFBP crypts resembled that seen at 3 weeks in IFCN.

PAPT/KOH/Bh/PAS

With this procedure, adjacent control sections which stained dark blue/purple indicated the absence of sialic acid residues. By 12 weeks of bypass, BPCN animals showed a variation in this staining, the predominant color now red/purple; reflecting an increase in O-acyl C_4 sialic acid substituents. Accordingly, while not prevalent, there were some sialomucins present that were masked in the KOH/AB1.0/PAPS technique. IFCN rats showed a more definitive red within the purple coloration; indicating the presence of sialic acid C_4 variants and those that are di- and tri-substituted. A similar staining pattern was exhibited by the IFBP group with a return to only a slightly red/purple stain (a decrease in substituted sialic acids) by the 12th week.

Transverse Colon

KOH/AB1.0/PAPS

Control crypts demonstrated a small number of basal, fuschia/purple staining cells; the rest being aqua/blue in coloration. This revealed some sialomucins within the basal regions despite a predominance of sulphomucins throughout the crypts. After 12 weeks in the BPCN group this staining pattern had not altered significantly. Inflamed controls exhibited an increase in red/pink staining (sialomucins) that extended from the base to one-third the way up the crypt. While an increase in sialomucin production was observed in the lower third, the upper two-thirds showed an abundance of sulphomucins (aqua). Both the IFCN and IFBP groups exhibited a normal histochemical staining pattern by the end of the experimental period.

PAPT/KOH/Bh/PAS

In control sections, the presence of blue/purple mucus cells demonstrated a lack of substituted sialic acids. Bypassing the normal colon did not influence this pattern appreciably. By contrast, IFCN crypts showed an initial increase in sialomucin-containing cells; with or without O-acyl substituents. As in the descending colon, sialomucin changes seen in IFBP crypts were not as marked as in inflamed controls.

Ascending Colon

KOH/AB1.0/PAPS

Sections taken from the normal ascending colon showed that mucus cells containing sulphomucins (aqua/blue) were localized in the upper half of the crypt, whilst those containing a mixture of both acid types (purple/fuschia) occupied the lower region. Adaptive hypoplasia in bypass controls was associated with not only fewer mucus cells but less epithelial mucins (a fainter stain). In this group moreover, cells in the basal crypt shifted from normal purple/fuschia to purple, reflecting an increase in sulphomucins at the expense of sialomucins. A similar mucin profile existed in IFBP crypts. IFCN glands, by contrast, displayed an increased number of sialomucin cells (red/pink) which had extended about two-thirds up the crypt.

PAPT/KOH/Bh/PAS

While the bottom half of control crypts contained a substantial amount of sialic acids that were at least substituted at C_9 (red/purple), the top half had none. With time, bypass (BPCN and IFBP) had resulted in reduced quantities of O-acyl sialic acid variants; basal crypt cells staining differently (purple/blue) from normal (red/purple). Such was not the case for inflamed controls which generally retained a normal pattern of mucin production.

V. DISCUSSION

Clinical investigations on the role of surgical bypass in the management of inflammatory bowel disease have yielded conflicting results (Lee and Harper 1983). The present animal study provided data on the influence of experimental bypass on acetic acid-induced colitis in male Sprague-Dawley rats. Results for epithelial morphometrics, kinetics, proliferation and mucus production all suggest that bypass has a protective effect against experimental inflammmation of the colon.

A. Morphology and Proliferation

Normal

The normal length of colonic crypts was similar to that described for Wistar/Furth rats by Lindstrom *et al.* (1979). Crypts in the transverse colon were longer than in the other two segments. In terms of cell units however, as earlier demonstrated in Sprague-Dawley rats (Tilson and Livstone 1980); crypts were universal in length with 32 cells. In slight contrast, the greatest number of mucus cells were observed in the proximal colon but fell off caudally. This was consistent with recent findings in adult Wistars that deep crypt mucus cells comprise respectively, 33%, 21% and 5% of ascending, transverse and descending colonic crypt cell population (Altmann 1983).

Total crypt cell population was similar in the ascending and transverse segments with 607 cells and was slightly higher in the descending with 677 cells. This reflected a proximal-to-distal increase in column count, in partial support of other rodent cytokinetic studies (Sunter *et al*. 1978, 1979).

In agreement with these studies also, cell proliferation was restricted to the lower two-thirds of the crypts. Futhermore, the segmental differences in proliferative zone height observed in this study support the observation that while most tritiated thymidine labelled cells are localized in the base of distal colonic crypts, they are found higher up the glands in the proximal segments.

Bypass-operated

It is well documented that the bypassed mucosa of the large bowel undergoes substantial atrophy, whereas restoration of bowel continuity with a return of intraluminal load reverses these changes (Rijke *et al*. 1979a; Terpstra *et al*. 1981; Delvaux *et al*. 1983, 1984). In the present study, prompt mucosal atrophy and hypoplasia occurred in the ascending and transverse colon from the first postoperative week. As the morphometric and cell count evaluations show, adaptation to bypass was most marked in the ascending colon, less in the transverse and virtually absent in the descending; suggesting a distance-related effect. A rapid and permanent shortening of ascending colonic crypts was characterized by a decrease in mean crypt depth which ranged from 51% to 71% of controls and in mean column height, ranging from 59% to 72%. Correspondingly, these values ranged from 76% to 89% and 72% to 84% in the transverse colon.

The hypoplasia induced; similar to that of previous experimental bypass studies on the colon (Rijke *et al.* 1979a; Delvaux *et al.* 1983) and small intestine (Rijke *et al.* 1977) of the rat, included a decrease in total crypt cell population, column length and column count. Particularly revealing however, was a reduction in the number of mucus-secreting cells in the longitudinal crypts.

This last finding was in contrast to the goblet cell hyperplasia shown in atrophic Thiry-Vella loops of the rabbit small intestine (Keren *et al.* 1975) and in the defunctioned small bowel of humans following jejunoileal bypass for morbid obesity (Asp *et al.* 1979); the former attributed to a local irritation in the bypassed loop. It was however, in keeping with the recent study of Olubuyide *et al.* (1984) which demonstrated that goblet cell hyperplasia is part of the adaptive response of the functioning small bowel of the rat to jejunoileal bypass. By inference, one might expect the the reverse response to bypass, namely a reduced number of mucus cells in the atrophic loop. Futhermore, in light of the proposed common origin of colonic epithelial cells from crypt-base stem cells (Chang and Leblond 1971), mucus cell hypoplasia should not at all be surprising.

The initial timing of the adaptive response seen in the ascending and transverse colon was consistent with the reports of Rijke *et al.* (1977) and Terpstra *et al.* (1981). The former also found that within 7 days, crypt and villus column heights of small intestinal Thiry-Vella fistula were substantially reduced in the rat. In addition, 1 week following transverse colostomy, the latter estimated a one-third reduction in defunctioned colonic cellularity. The magnitude of crypt shortening decribed in this work was reminiscent of the 49% shorter crypt column seen in the colon of elemental diet-fed rats (Janne *et al.* 1977). The feeding regimen however, induced more marked changes in total crypt cell population than did colonic bypass; compared to normal, crypts were decreased to 25% by the former method and to 41% (ascending) and 61% (transverse) on average, by the latter.

Except for a significant reduction during the first postoperative week, bypass had no clear-cut effect on the cell population of the distal colon. The transitory hypoplasia could probably have resulted from the stress of operative trauma (Williamson and Malt 1980a). Futhermore, the simultaneous rise in mitotic number was previously described within 12 hours to 2 weeks of wounding in the epithelial cells of rectal mucosa (Reeve 1974). An overall lack of adaptation to bypass could possibly be due to a combination of factors. First, if, as has been suggested (Williamson 1982; Delvaux *et al.* 1983), colonic hypoplasia results primarily from a loss of mechanical stimulus provided by fecal bulk; the reflux of material from the functioning bowel into the blind loop might conceivably have prevented or reversed the anticipated hypoplastic response (Solhaug 1976). Such regurgitation was observed in this and previous animal studies (Shute and Bombeck 1975).

Second, bypass has been implicated in a localized hypertrophy observed around the anastomotic site in rats (Nygaard 1970). Finally, such hypertrophy might be related to the temporary hyperproliferative response elicited at day 30 by transection and anastomosis in the normal colon of male Sprague-Dawley rats (Tutton and Barkla 1983) although not all studies are in agreement (Delvaux *et al.* 1983).

The data for crypt height correlate well with those from the few experimental bypass colonic studies. In one such study on male Sprague-Dawley rats, tissue samples transected on the 15th postoperative week from the splenic flexure of Thiry-Vella fistulae, showed a significant reduction in crypt depth. A decrease in crypt height expressed in terms of cell units, has also been described in the defunctioned distal colon of rats with transverse colostomies; at 3 weeks by Delvaux and co-workers (1983) and at 6 weeks by Rijke and his associates (1979a). The rapid onset of crypt column shortening seen within 7 days both in this study and that of Rijke *et al.* (1977) was at least two weeks earlier than that reported by the above studies. However, Rijke *et al*. studied adaptation to bypass at one period of time (6 weeks) only, whereas Delvaux *et al*. did so at 5 days and at 3 weeks. Moreover, within just 5 days, the latter workers had reported significant hypoproliferation; indicated by substantial decrease in mitotic and labelling indices. It is conceivable therefore, that a similar temporal onset of shorter crypts could have been observed in the defunctioned colon , had Delvaux *et al*. (1983) employed closer and different sacrifice intervals.

The basic difference between the alterations observed in this and other colonic experimental bypass studies (Rijke *et al*. 1979a; Delvaux *et al*. 1983) was found in the differential segmental adaptation described herein. This discrepancy might be the result of a difference in experimental models. The blind loop created by an end-to-side anastomosis is not strictly comparable to Thiry-Vella fistulae or colostomies. Creation of the last two involves transection of the defunctioned segment from the rest of the large bowel, thus negating any possibilty of voluntary retrogade reflux. The distance-related adaptative capacity associated with the isoperistaltic blind loop was also demonstrated by Fenyo and Hallberg (1976) in the small bowel.

The progressive mucosal atrophy observed with increasing distance from the anastomotic site and the normoplastic appearance of the distal colon seem to support, at least in part, the concept that colonic architecture is maintained primarily by the mechanical stimulus provided by fecal bulk (Stragand and Hagemann 1977; Ryan *et al.* 1979; Williamson 1982; Delvaux *et al.* 1983; Menge and Robinson 1983; Rainey *et al.* 1983). Contributing to the interpretation of these findings were the following observations made on the colon of the rat: the severe mucosal atrophy promoted by transverse colostomy and its reversal following bowel closure (Terpstra *et al.* 1981; Delvaux *et al.* 1983); the gastrin-independent postprandial rise in proliferative activity (Majumdar 1984); the significant mucosal atrophy elicited by enteral and parenteral bulk-reducing diets (Janne *et al.* 1977; Ryan *et al.* 1979; Morin *et al.* 1980; Storme and Willems 1981; Heitman *et al.* 1983) and the reversal of this atrophic effect in the absence of gastrin, by the addition of non-absorbable bulk to the liquid diet (Ryan *et al.* 1979).

In five of these studies (Janne *et al.* 1977; Ryan *et al.* 1979; Morin *et al.* 1980; Storme and Willems 1981; Heitman *et al.* 1983), elemental diets induced colonic atrophy within 6 days. This was reflected by significant reductions in crypt cellularity, column length, column count, colonic weight, mucosal DNA and protein contents, proliferative zone size, and labelling and mitotic indices.

Differential, distance-related segmental adaptation may also be supported by the results of fast-feeding experiments. Food deprivation induced colonic atrophy and depressed proliferative activity, but refeeding of chow pellets led to reversal of these changes. (Hagemann and Stragand 1977; Majumdar 1984). More importantly however, was that the latter occurred without a rise in serum and tissue gastrin levels (Majumdar 1984).

In the presence of mucosal atrophy as described for the ascending and transverse colonic segments, permanent, substantial, proportional decreases were demonstrated in the number of mitotic figures, proliferative zone size and height, the average height of mitotic figures and the percentage of mitotic cells per crypt column (mitotic index). This strong bypass-induced trophic effect sheds additional light on the hypoproliferative mediation of the cell kinetic expression to reduced intraluminal load (Janne *et al.* 1977; Storme and Willems 1981; Delvaux *et al.* 1983, 1984).

In contrast to the results reported by one earlier study (Rijke *et al.* 1979a), these observations were similar to those of Delvaux *et al.* (1983), who reported a similar timing of the initial hypoproliferative response. They showed that diverting colostomy led to a significant decrease in labelling index, mitotic index and DNA synthesis in both the distal defunctioned and proximal functioning colon; in the case of the former, by the 5th postoperative day.

These findings also correspond with those of Storme and Willems (1981) who, within 6 days of an elemental diet, reported shorter proliferative zone and depressed proliferative indices in the distal and transverse colon of male Wistar rats. The magnitude of hypoproliferation also reflects the 50% less RNA, DNA and labelling indices associated with both colostomies (Terpstra *et al*. 1981) and liquid diets (Janne *et al*. 1977).

As suppressed cell turnover was commensurate with differential segmental morphological and cell kinetic changes, hypoproliferation was maximal in the proximal colon, less in the middle and virtually absent in the distal.

Inflamed Only

Treatment of Sprague-Dawley rats with 10% acetic acid resulted in prompt morphological and cell kinetic changes in mucosal crypts of the distal and middle colonic segments within 7 days; about 4 to 6 days after ulcers first appear (MacPherson and Pfeiffer 1978; Mann *et al*. 1980). Proximal (with respect to acetic acid application site) response was greater than distal. Consequently, hypertrophy and hyperplasia were maximal in the descending colon, less in the transverse, and minimal in the ascending where it was delayed 1 week.

Crypt length assessed both morphometrically and in terms of cell units as well as mucosal height and crypt size peaked at week 1 in the descending colon and at week 3 in the transverse; probably reflecting a distance-related response. In terms of crypt length, mean column length in the latter increased by a factor of 1.3, from 32 cells in control crypts to 43 cells in IFCN crypts; while that for the former increased by a factor of 2.2, from 32 to 70 cells. Similar values to those seen in the distal colon were observed in the hyperplastic glands of patients with coeliac disease which is noted for its exaggerated cell turnover (Croft and Cotton 1973; Watson *et al.* 1982). Proportional increments in the morphometric values were indicated by percent values of 198% and 128% for crypt depth of the descending and transverse colon, respectively. This was expected (Wright 1982) and confirmed by the high positive correlation coeficient (r=0.95) between these two estimators of crypt length.

Crypt size enlarged 2.2 times (1493 IFCN cells vs 677 control cells) in the descending colon and 1.4 times (849 vs 607) in the transverse. The hyperplasia induced was substantiated in a preliminary study (Pon *et al.* 1982) and was consistent to with those demonstrated in carrageenan colitis (Watt and Marcus 1970), DMH-treated mouse colon (Richards 1981), ischemic colitis (Franzin *et al.* 1983), quiescent ulcerative colitis (Franzin *et al.* 1983) and the non-polypoid mucosa of human colonic diverticula disease (Rhatigan 1979). Also similar to this study, is the fact that the last two included an increase in the number of mucus (goblet) cells. Acetic acid-induced mucus cell hyperplasia agrees with that shown during the post-irradiation recovery of the small intestine (van Dongen *et al.* 1976) and thus appears to be a general mucosal expression to irritation and injury (Keren *et al.* 1975).

Following each segmental peak, values for these parameters fell gradually and reverted to normal by week 8. In accordance with Mann *et al.* (1980) and contrary to the transitory response exhibited by the ascending colon, inflammatory changes persisted in the rest of the large bowel for about 8 weeks at which time lesions have healed both endoscopically and histologically.

Unlike crypt length, no clear-cut differences in crypt width and column count were found between inflamed and normal controls; although a slight insignificant increase might have occurred at week 2. As a consequence, there was no change in the number of transversely cut glands per unit area. These findings, interestingly, were opposite to those reported for coeliac and DMH-induced hyperplasia (Richard 1981; Watson *et al.* 1982) and for the hyperplastic villi of self-emptying jejunal blind loops (Menge *et al.* 1979). However, in the DMH colon, a significant increase in column count was recorded only after 8 weeks of chronic treatment (Richard 1981).

The hypertrophy and hyperplasia caused by the cytotoxic insult, were paralleled by proportional increments in crypt cell proliferation. This resembled the adaptive changes seen in residual intestine after resection (Hanson *et al.* 1977) and in the mucosa from different stages of coeliac disease

(Watson *et al.* 1982). These results were also in agreement with the proliferative response to acid-induced mucosal injury seen in duodenal ulcer and duodenitis (Bransom *et al.* 1984) and to those of DMH- and irradiation-induced alterations of the mouse colon (Richards 1981; Richards and Tacha 1982).

Exaggerated cell turnover in the descending colon of inflamed controls was indicated by a rapid, initial near doubling (or near trebling) of the size of the proliferative zone; the average height of mitotic figures along the crypt column; and by the frequency of mitotic figures seen in the progenitor area. These alterations coincided with sharp rises, twice above normal, in crypt length, mucosal height and total crypt cell population; features of the general response to colonic toxicity (Barthold 1981).

The observations made favor the hypothesis that hyperproliferation and hyperplasia are compensatory, reparative responses to excessive injury-induced cell losses from the mucosal surface into the gut lumen (Barthold 1981; Richard 1981; Watson *et al.* 1982; Bransom *et al.* 1984) and that the degree of compensation is correlated with the extent of cell depletion (Richards and Tacha 1982). Richards (1981) and Barthold (1981) showed that in DMH-induced colonic hyperplasia in mice, chronic DMH treatment resulted in progressive elongation of crypt cell column, increased labelling indices and expansion of the proliferative zone. Richards suggested that taller crypts probably resulted from cell production in the crypt exceeding cell loss at the surface; thus implying that the normal steady state (Eastwood 1977) had been disturbed. Others, however, have implied that longer crypts can result from a new steady state in which increased cell production is balanced by an equal increase in cell loss (Barthold 1979; Menge *et al*. 1979; Watson *et al* 1982). Such might have been the case in this study as there seemed to be no change in the relative size of the proliferative zone.

Both estimators of proliferative zone height showed the same general response pattern to inflammation. However, mean highest mitotic figure was slightly lower than the mean leading edge. Two reasons may be given for this; actual and potential errors inherent in the ^{3}HTdR labelling technique (Maurer 1981; Wright 1982) and the share difference in the number of S phase cells compared with mitotic figures seen in the progenitor area of the crypt; the former exceeding the latter about 20 times (Willems 1979).

Although there was an increase in proliferative zone height, there appeared to be none in the relative size of this compartment. The fact that proliferative zone height extended in proportion to the absolute height of the crypt could account for this observation, which in turn, might represent a new state of equilibrium reached between cell loss and cell production. Alternatively, no change in the absolute size of the proliferative zone might reflect the sampling procedure as varying degrees of mucosal damage are induced by the inflammatory agent. Indeed, labelled cells and mitotic figures were sometimes seen in the upper third of crypts adjacent to the site of ulceration. Expansion of the proliferative zone from the lower two-thirds of the crypt to the upper third, in contrast to these results, have been demonstrated in the hyperplastic or dyplastic colonic mucosa and polyps of Crohn's disease (Shannon *et al.* 1981), ulcerative colitis (Biasco *et al.* 1979; Butt *et al.* 1983), colorectal carcinoma (Stoffels *et al.* ; Deschner and Maskens 1982) and DMH-treated rodents (Richards and Tacha 1982).

Inflamed-operated

The cumulative evidence provided by this study suggest a protective role for bypass against acetic acid colitis. Within a week of exclusion from the fecal stream, values for most parameters in the inflamed-operated group were significantly reduced compared to inflamed controls. At 1 week; column length, crypt depth, total crypt cell population, and the height of the proliferative zone estimated from the leading edge, for the descending colon were respectively, 30%, 20%, 25% and 54% below inflamed controls. Alternatively, they were 46%, 44%, 59% and 39% above bypass controls. The relative decrease from inflamed values was generally maintained from week 1 to week 4. By week 8, however, the gap between IFCN and IFBP values had disappeared.

It is noteworthy that for several parameters, IFBP values at week 4 were equivalent to IFCN values at week 8. For instance, mean column length and crypt depth for IFBP crypts at 4 weeks were respectively, 38 cells and 288.8 micrometers; whereas corresponding values for IFCN crypts at week 8 were 36 and 283.6. Similarly, crypt cellularity (745 cells) at 4 weeks was equal to IFCN value (752) at 8. A similar trend was observed for proliferative zone height except that IFBP values at week 4 was now equal to IFCN values at week 10. These findings suggest that in spite of the periodic flare (week 6), bypass had resulted in a near-normal recovery from the acetic acid-induced injury of the descending colon within 4 weeks; that is, 1 month before the inflammatory process had ended in the inflamed controls.

The irregular IFBP values exhibited by the week 6 rats could reflect the fact that a different group of animals was used for this interval only. Another possibility could be its is relationship to a recently described non-specific colitis induced by fecal diversion in the defunctioned colon of patients with non-inflammatory bowel disease (Glotzer *et al*. 1981; Bosshardt *et al*. 1984). Diversion colitis remains unexplained. Why this irregular finding occurred in this group of rats only is unclear and might help to explain why some surgeons (Harper *et al*. 1983) report satisfactory results following fecal diversion for inflammatory bowel disease while others (Jones *et al*. 1966) do not.

From the morphometric, epithelial counts and proliferative data for the first 4 weeks, IFBP crypts appeared to be in a hyperplastic and hyperproliferative state that was to a greater or lesser extent intermediate between IFCN and BPCN glands. This implies a direct influence of bypass on crypt cell kinetics in acetic acid colitis which, in turn, might be indicative of a bypass-induced depression of the inflammatory activity during the process of recovery from the inflamed mucosa. This is analagous to the regenerative stage-dependent and activity-related variations in hyperproliferation and hyperplasia which have been observed in ulcerative colitis (Biasco *et al.* 1979) and coeliac disease (Watson *et al.* 1982).

With respect to ulcerative colitis, rectal biopsies from patients with severe UC are in a higher state of proliferation than from those with a slight form of the disease. The latter in turn, is higher than in patients within the quiescent phase. (Biasco *et al.* 1979). Similarly, in the case of coeliac disease of the small intestine, flat mucosa is more hyperplastic and hyperproliferative than group-3, convoluted, remission mucosa which exhibits a higher cell turnover than normal mucosa (Watson *et al.* 1982).

It is interesting to note that the convoluted mucosa of coeliac disease represents a stage (intermediate) in the recovery from flat mucosa. Similarities between this human example and this experimental model are very striking. In the study of Watson *et al.* (1982), flat mucosal crypt column length was 75 cells as opposed to the 70 cells for IFCN crypts. Convoluted mucosal crypts were 39 cells long, while IFBP crypts were 54 cells in length. Moreover, both normal mucosa and control glands were equal in length, with 32 cells. From these stage-dependent variation in crypt column length in addition to epithelial cell proliferation, it is reasonable to assume that inflamed-bypass mucosa, similar to convoluted, remission mucosa, demonstrates a recovery from that of inflamed controls.

Clinical studies support the bypass protective effect. Several series have reported that ileostomies for Crohn's colitis resulted in immediate symptomatic relief as well as marked improvement of local lesions (Oberhelman *et al.* 1968; Burman *et al.* 1971; Harper *et al.* 1983; Lee and Harper 1983). At least two (Burman *et al.* 1971; Lee and Harper 1983), claimed healing of some fissures and fistulae. However, histological signs of residual inflammation in the isolated colon indicate that complete subsidence and healing of the disease are rare (Harper *et al.* 1983). The same protection also occurred following "medical bypass" of the inflamed colon with a liquid elemental diet (Giorginie *et al.* 1973).

The protective role of bowel rest observed in this study was further supported by that against chemical carcinogenesis in Sprague-Dawley rats following colonic bypass (Williamson and Malt 1980; Rainey *et al.* 1983). Similarly, total parenteral nutrition was found to have a protective effect against DMH-induced colonic hyperplasia and hyperproliferation (Heitman *et al.* 1983). The reductive effect of Thiry-Vella fistulae against experimental colonic cancer was ascribed by Rainey *et al.* (1983) to the mucosal hypoplasia of the defunctioned large bowel that stemmed from a loss of mechanical stimulus concomitant with reduced intraluminal load. Yet, others have attributed it to biliary diversion away from the colon (Navarette and and Spjut 1967). Moreover, Heitman *et al.* (1983) have implied that chemical makeup of luminal content is just as important as its physical form; since an elemental diet does not have the same effect on DMH hyperplasia as total parenteral nutrition.

The possibility that recovery from acetic acid colitis might also have been triggered solely by topographical, bulk reduction-induced hypoplasia has to be considered; particularly for the transverse colon. However, in the absence of any significant mucosal atrophy in the distal segment of bypass controls (except at 1 week); the question arises as to how bypass could have caused such reductive inflamed changes? Perhaps both local and systemic factors are involved in the regenerative process as Williamson suggests (1978b). Several mechanisms could explain these observations.

Proximal adaptation in the ascending and transverse segments, might have affected the the distal inflamed colon by indirect means. There is accumulating evidence that at least a part of the adaptive response to colonic bypass is humorally mediated (Delvaux *et al.* 1983, 1984). Indirect effects of bypass is substantiated by one recent study (Delvaux *et al.* 1983) showing decreased epithelial cell renewal in both normal appearing, proximal, functioning and atrophic, distal, defunctioned segments following transverse colostomy. Similar indirect influences were described for parenteral nutrition (Dworkin *et al.* 1976).

Another possibility might be the "subsiding effect" promoted by diverting bile and other deleterious substances away from the inflamed mucosa (Attisha 1982). Such an assumption would be in keeping with the theory that colostomy-induced protection against chemical carcinogenesis in rats is provoked not only by bile acid diversion but also by alterations in bile acid and bacterial population of the distal colon (Navarette and Spjut 1967; Campbell *et al.* 1975; Terpstra *et al.* 1981). Bile is known to have a powerful trophic effect on colonic mucosa (Deschner 1980; Deschner *et al.* 1981), partly due to its chronic irritation (Williamson 1978b). In rats, bile deprivation induces colonic hypoplasia and hypoproliferation (Deschner and Raicht 1979). By contrast, sulphated bile acid colonic perfusion elicits inflammatory changes (Breuer *et al.* 1983). These findings suggest that while the presence of bile could aggravate acetic acid colitis, its absence might very well alleviate this condition. Less inflammation resulting from biliary diversion concomitant with surgical bypass, would be opposite to, but in line with, the cholic acid enhancement of chemical carcinogenesis in rats (Cohen *et al*. 1981); attributed to increased proliferative activity provoked by mucosal bile injury (Deschner *et al*. 1981).

Changes in colonic microflora subsequent to intestinal bypass (Bjorneklett *et al*. 1981), could also explain this bypass effect and may be related to a reduction in "physiologic inflammation" (Williamson 1978a). Broadly similar, is the delay in post-irradiation mitotic increase observed in the hypoplastic mucosa of germ-free rats (Galjaard *et al*. 1972; Williamson 1978b).

B. The Pattern of Mucus Production

Normal

In this, as well as in one previous study (Olubuyide *et al*. 1984)on male Sprague-Dawley rats, there was a predominance of sulphomucins throughout the normal colon; similar to that described in male Fischer 344 (Shamsuddin and Trump 1981), female Wistars (Filipe 1975) and humans (Filipe and Branfoot 1976; Franzin *et al*. 1983; Reid *et al*. 1984b). The KOH/AB1.0/PAPS histochemical stain confirmed that crypts of the normal distal colon contain a predominance of sulphomucins (Filipe 1975; Olubuyide *et al*.

1984), whereas those of the proximal segment have sulphomucins in the upper half with a mixture of both acid mucins in the lower region. In addition, this stain revealed that transverse colonic crypts also contain an abundance of sulphomucins in spite of few basal sialomucin-containing cells. It appears therefore, that although the normal rat colon exhibits sulphomucin predominance, a distal-to-proximal sialomucin gradient exists. Similar to other studies (Reid *et al* 1975, 1977), more sialic acid substituents were located in the upper colon compared to the lower as shown by the PAPT/KOH/Bh/PAS method.

Bypass Controls

Bypassing the colon (BPCN) resulted in different segmental changes in the normal distribution of acid mucins; providing further evidence of the varied segmental adaptive capacity associated with anastomotic colonic bypass. In the region of the defunctioned loop furthest away from the anastomotic site, residual mucins were characterized by an increase in sulphomucins at the expense of substituted and unsubstituted sialomucins. Just the opposite was shown in the functioning hyperplastic bowel following jejunoileal bypass in rats (Olubuyide *et al*. 1984).

The basis for the reduced levels of sialomucins may be two-fold. On the one hand, it could be a histochemical expression of the hypoplastic and hypoproliferative changes characteristic of the atrophic colon as the reverse situation (sialomucins excess) appears to be associated with hyperproliferative conditions. These include bypass-induced hyperplasia (Olubuyide *et al.* 1984), transitional and regenerative mucosa (Franzin *et al.* 1983; Ehsanullah *et al.* 1985), in addition to early fetal colonic mucosa (Filipe and Branfoot 1976). On the other hand, if, as suggested by Filipe and Branfoot (1976), sialomucins are primarily protective, whereas sulphomucins are lubricative; it follows that less of the former would be produced by the ascending colon since it is no longer exposed to the deleterious effects of fecal content.

In contrast to the ascending colon, a slight increase in sialomucins was detected in the distal colon; with the PAPT/KOH/Bh/PAS method only. This observation is consistent with the abundant sialomucins recently demonstrated in the vicinity of the anastomosis after jejunoileal bypass (Olubuyide *et al.* 1984). Given the shortness of the distal colon of the rat, tissue samples from this region would have been resected from fairly close to the anastomotic site. According to Olubuyide *et al.* (1984), this abnormal mucin pattern probably represents a protective response to local operative trauma and fecal stasis. As discussed earlier, voluntary, retrograde reflux is not uncommon in this type of experimental bypass.

The relatively normal mucin pattern persisting in the transverse colon is substantiated by the finding that bypass-induced sialomucin excess tapers off with increasing distance from the site of anastomosis (Olubuyide *et al*. 1984). This might reflect the extent of the bypass adaptive response observed in this segment compared to the other two.

Acetic Acid-Treated Animals (IFCN and IFBP)

There is a general agreement in the literature that colonic inflammation results in a shift toward sialomucin production (Filipe and Branfoot 1976; Filipe 1979; Reid *et al*. 1984-; Ehsannullah *et al*. 1985). This pattern was also seen in this and other studies on acetic acid colitis (MacPherson, Reid and Tabor 1985). That sialomucin predominance was evident in all three regions of the inflamed controls, seems to suggest that the entire colon is responsive to the non-specific inflammation induced by acetic acid. However, the significant sialomucin excess demonstrated in the descending and transverse regions compared to the ascending, indicates that this response might be positively correlated with the degree of mucosal contact with the inflammatory agent.

Sialomucin response which included an increase in its O-acyl substituents; particularly the C_9 variant, can occur as early as 30 minutes following acetic acid administration (MacPherson *et al*. 1985) and may serve as a protective mechanism against injury and irritation (Filipe and Branfoot 1976).

Acetic acid-induced sialomucin predominance is analogous to similar increases shown in ulcerative colitis

(Franzin *et al*. 1983; Ehsanullah *et al*. 1985), ischemic colitis (Franzin *et al*. 1983), inflammatory and hyperplastic polyps (Franzin *et al*. 1983), and the hyperplastic bowel of the rat after intestinal bypass (Olubuyide *et al*. 1984); these conditions sharing the common feature of exaggerated cell turnover.

The reasons for this abnormal pattern of mucin production is debatable. Some suggest that it might be a non-specific reaction, possibly regenerative, which regresses when inflammation terminates (Franzin *et al*. 1983; Ehsanullah *et al*. 1985). Yet others have advanced the hypothesis that it is associated with precancerous mucosa (Filipe 1979; Ehsanullah *et al*. 1982, 1985). In general agreement with the first suggestion, sialomucin production was shown to be substantially reduced in the inflamed-bypass group compared to inflamed controls. Furthermore, a return to a normal sulphomucin predominance was evident by the time the inflammatory process had ended. Consequently, similar to clinical studies (Ehsanullah *et al*. 1982, 1985), the relative proportion of the two acid mucins seems to be related to the activity , extent and duration of acetic acid colitis.

These findings do not preclude the possibility that this mucin profile may be indicative of precancerous changes. Acetic acid colitis (Hagihara 1982) as well as jejunoileal bypass (Bristol and Williamson 1981) have been shown to enhance chemical carcinogenesis in rats, while long-standing ulcerative colitis predisposes man to colonic tumors (Riddell 1976). Moreover, it has recently been reported that sialomucin excess is highly correlated with the dysplastic mucosa of ulcerative colitis (Ehsanullah *et al.* 1985).

Although both inflamed control and inflamed-bypass animals showed a predominance of sialomucins, a substantial reduction in this acid mucin was evident in the latter compared to the former within 1 week of bowel rest; further substantiating the protective role of experimental bypass against experimental colitis. Interestingly, in the case of the descending colon, the abnormal mucin profile seen at week 1 for the inflamed-bypass group resembled that at week 3 for inflamed controls. Also, the shift towards sialomucin production seen in the ascending colon of inflamed controls was opposite to the selective decrease in this acid mucin demonstrated in the corresponding segment of the inflamed-operated group. Bypass-induced regression to a normal mucin pattern is entirely consistent with those clinical studies which show that ulcerative colitic mucosa in remission is predominantly sulphomucin in character and that increased sialomucin production is dependent on the disease index (Ehsanullah *et al.* 1982, 1985).

Limitations of the Methodology

The foregoing study has focused on the colonic response to bypass and acetic acid toxicity using primarily morphological and cell kinetic methods, "two of the mainstays of the study of intestinal adaptation" (Wright 1982).

For morphological assessment, the available methods were the extremely time consuming cell counts and morphometry of histological sections as opposed to other more accurate techniques proposed by Wright in 1982.

Inherent in the use of indirect estimators are a number of deficiencies which include imprecision, counting errors and changes related to fixation and sectioning (Wright 1982). It has been argued that indirect estimators such as villus row count and crypt column count, in the case of the small intestine, are not accurate measurements of cellularity unless they have been proven to be well correlated with the total number of cells within the villus and crypt compartments (Wright 1982). It has further been suggested that product estimators (e.g. total crypt cell population), which result from combining length (column height) and width (column count) estimators, can provide a much more definitive estimation of crypt cellularity.

Despite the limitations that might be associated with the use of indirect estimators, meaningful inferences can be drawn from the results of this study especially given the high cummulative Pearson correlation coefficients (Table IV.4) between many of the variables (Wright 1982). These include: crypt depth and column height (r=0.95); crypt width and column count (r=0.87 and r=0.83); column count and total crypt cell population (r=0.97); crypt depth and total crypt cell population (r=0.91); proliferative zone and column heights (r=0.85); and total crypt cell population and the height of the proliferative compartment (r=0.83).

Similar estimators to those used in the present study have also been employed in recent colonic studies on intestinal adaptation (Sunter *et al.* 1978, 1979; Rijke *et al.* 1979a; Storme and Willems 1981; Heitman *et al.* 1983; Rainey *et al.* 1983; Delvaux *et al.* 1983, 1984).

With respect to cell proliferation, Maurer (1981) has discussed a number of pitfalls associated with the ^{3}H-thymidine technique; including factors related to the metabolism of tritiated thymidine itself. A more reliable method is said to be the proportion of cells in mitosis (Wright 1982), for example, the mitotic index which can be expressed as the percentage of metaphase figures per total number of longitudinal crypt cells (Heitman *et al.* 1983). However, the number of mitotic figures cells present in the proliferative compartment of the crypt at any one time is very small (Willems 1979).

These limitations restrict the conclusions that can be drawn from the proliferative data. Nevertheless, in the case of the leading edge for instance, although this parameter by itself cannot provide a definitive index of cell proliferation, the positive correlations between it and, (1) the number of mitotic figures (r=0.66); (2) the average height of mitotic figures (r=0.71); (3) total crypt cell population (r=0.83); and (4) crypt height (0.83) do imply that fairly accurate inferences can be drawn from the proliferative data.

Clinical Significance of the Findings

Acetic acid colitis in rats shares some histopathological features with human colitis (Sharon and Stenson 1985). If the results of this controlled, animal study can be extrapolated to humans, then anastomotic bypass may have an important place in the surgical management of inflammatory bowel disease. In some cases, it might serve as a welcome alternative to colectomy with ileostomy (Johnson *et al.* 1982), particularly in the young. The advantages are that bowel is conserved and the normal route of evacuation maintained.

VI. SUMMARY AND CONCLUSION

The purpose of this investigation was to assess the influence of surgical bypass on experimental colonic inflammation in male Sprague-Dawley rats. As documented in Chapter IV, the cell counts and morphometric evaluations showed that the majority of the morphometric, quantitative and proliferative parameters exhibited a characteristic pattern of response to 10% acetic acid. Crypt depth, crypt height in number of cells, mucosal height, the number of mucus cells per longitudinal crypt, total crypt cell population and most proliferative parameters showed an immediate 2-fold increase ($P < 0.05$) in the descending colon and a 1.5-fold increase in the transverse following acetic acid treatment, but then gradually decreased to normal levels; by 8 to 10 weeks in the former and by 6 weeks in the latter. Peak values generally occured at week 1 in the descending colon and at week 3 in the transverse. Luminal expression to acetic acid toxicity followed a similar trend. After a delayed reaction, the ascending colon, in slight contrast, demonstrated insignificant increases at week 2 and subsequently normalized at week 8.

In contrast to the inflammatory response, fecal diversion in the bypass control group led to substantial ($P < 0.05$) and permanent decreases in these parameters; in ascending and transverse colonic segments but not the descending. These results suggested that the adaptive capacity of the rat colon to colocolic bypass was distance-related and at least dependent on the absence of fecal bulk.

Fecal diversion had a significant influence ($P < 0.05$) in depressing the inflamed values of all variables. Compared to inflamed controls, in the inflamed-bypass group, IFCN values were abolished in the proximal colon with an early return to normalcy in the other two segments; by 2 weeks in the transverse and by 4 weeks in the descending. Taken together, these results indicate that fecal diversion had a reductive effect on the colonic mucosal changes associated with acetic acid colitis and that both local and systemic factors might have been involved in this protective mechanism.

The histochemical study showed that sections of the transverse and descending colon from IFBP animals stained less intensely for sialomucins, substituted and unsubstituted, than those from the IFCN group. This was evident within 1 week of bowel rest, that is, at the same time as the other bypass-reduced inflamed changes. Fecal diversion, moreover, led to an increase in sulphomucins at the expense of sialomucins in the atrophic proximal colon. Since bypass reversed the shift towards sialomucin which is characteristic of active inflammation (Ehasannulah *et al*. 1985), these results further confirmed the reductive effect of fecal diversion.

In summary, it can be concluded that colocolic bypass has a protective effect against experimental acetic acid colitis in male Sprague-Dawley rats. It is conceivable, therefore, that bowel rest might play an important role in the surgical management of human inflammatory bowel disease.

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Appendix 1

Microscopic Fixation and Processing Procedure

Neutral-Buffered Formalin (10%)

Formalin	1000.0 ml
Sodium acid phosphate ($NaH_2PO_4.H_2O$)	4.0 g
Anhydrous disodium phosphate (Na_2HPO_4)	6.5 g

(a) Methacrylate Tissue Processing

1.	10% neutral-buffered formalin	24 h
2.	70% alcohol	1 h
3.	85% alcohol	1 h
4.	95% alcohol (2 changes)	30 min each
5.	Catalysed solution A:95% alcohol (1:1)	1 h
6.	Catalysed solution A:95% alcohol (1:3)	30 min
7.	Straight solution A	overnight

8. Place oriented tissue in mould with embedding mixture (A+B).

Catalyzed Solution A (Infiltration Solution)

Solution A (JB 4)	100 ml
Catalyst	0.9 g

Mix until dissolved.

Embedding Mixture

Catalysed solution A	25 parts
Solution B	1 part

Stir rapidly and thoroughly.

Paraffin Tissue Processing (Automatic Processor)

1.	50% alcohol	2 h
2.	60% alcohol	2 h
3.	70% alcohol	2 h
4.	80% alcohol	2 h
5.	95% alcohol (2 changes)	1 h each
6.	Absolute alcohol	1 h
7.	Absolute alcohol	1 h
8.	Absolute alcohol	30 min
9.	Choloform or xylene (2 changes)	1.5 h each
10.	Paraffin infiltration (2 changes)	45 to 60 min each

11. Embed tissue in paraffin.
12. Cut tissue at 6 um.

Appendix 2

Lee's Methylene Blue - Basic Fuchsin (MBBF)

SOLUTIONS

Methylene Blue Stock

Methylene blue	0.5 g
Distilled water	400.0 ml

Basic Fuchsin Stock

Basic fuchsin	0.5 g
Distilled water	400.0 ml

Stock Buffer

Solution A:

Sodium phosphate monobasic, monohydrate	27.5 g
Distilled water	1000.0 ml

Solution B:

Sodium phosphate, dibasic, 12-hydrate	71.5 g
Distilled water	1000.0 ml

Stock buffer pH 6.8 to 7.2. Higher pH levels produce bluer results. Lower pH values give pinker results.

Working Solution

Methylene blue	12 ml
Basic fuchsin solution	12 ml
Solution A (Stock buffer)	7 ml
Solution B (Stock buffer)	18 ml

Ethyl alcohol, 95% .... 15 ml

Filter.

STAINING PROCEDURE:

1. Stain in working solution for 2 min.
2. Rinse in distilled water.
3. Dip in 95% alcohol to remove background stain (2 dips).
4. Blow dry.
5. Mount.

Appendix 3

Periodic Acid-Schiff - Alcian Blue (pH 1.0 or 2.5) Staining Procedure

1. Deparaffinize and hydrate to distilled water.
2. 1% alcian blue solution adjusted to 2.5 (1.0) for 30 min.
3. Blot section with filter paper if 1.0 is used; wash in water for 5 min if AB 2.5 is used.
4. Oxidize in periodic acid solution for 10 min.
5. Wash in running water for 5 min.
6. Schiff reagent for 10 min.
7. Rinse in sodium metabisulfite solution for 2 min/3 changes.
8. Wash in running water for 10 min.
9. Dehydrate in 95% alcohol; 100% alcohol and xylene (2 changes each).
10. Mount in permount.

1% Alcian Blue Solution

pH 2.5:	Alcian Blue 8GX	1 g
	Glacial acetic acid, 3%	100 ml
	Adjust the pH to 2.5 and add a few crystals of thymol.	
pH 1.0:	Alcian blue 8GX	1 g
	HCl, 0.1N	100 ml

1% Periodic Acid solution

periodic acid	1 g
distilled water	100 ml

0.5% Sodium Metabisulfite Solution

sodium metabisulphite	0.5 g
distilled water	100 ml

Appendix 4

The PAPT/KOH/Bh/PAS Histochemical Staining Procedure (Reid *et al*. 1984c)

1. Bring sections to water.
2. Oxidize with 1% periodic acid. The period of oxidation used must be established in preliminary studies and must be sufficiently prolonged to give a negative PBT-PAS reaction.
3. Wash in running water for 5 min.
4. 0.5% (w/v) aqueous phenylhydrazine hydrochloride for 2 h at room temperature.
5. Distilled water for 16 h.
6. Rinse in 70% ethanol.
7. Saponify with 0.5% KOH in 70% ethanol for 15 min at room temperature.
8. Wash for 5 min in running water.
9. Reduce with 0.1% (w/v) sodium borohydrate in 1% anhydrous dibasic sodium phosphate for 30 min at room temperature.
10. Wash in running water for 5 min.
11. Oxidize with 1% periodic acid for 5 min.
12. Wash in running water for 5 min.
13. Stain with freshly prepared Thionin Schiff reagent for 30 min.
14. Wash in running water for 10 min.
15. Dehydrate, clear and mount.

1.0% Periodate:

Dissolve 1 g of periodic acid in 100 mls of H_2O

.

0.5% KOH:

Dissolve 0.5 g KOH in 100 mls 70% EtOH.

0.5% Phenylhydrazine-HCl:

Dissolve 0.5 g phenylhydrazine-HCl in 100 ml H_2O.

0.1% $NaBH_4$:

Dissolve 1 g Na_2HPO_4 in 100 mls H_2O. Then dissolve 0.1 g $NaBH_4$ in the 1% Na_2HPO_4.

Appendix 5

The KOH/AB1.0/PAPS Histochemical Stain

1. Saponify with 0.5% KOH in 70% (w/v) aqueous ethanol for 15 min at room temperature.
2. Stain with Alcian Blue pH 1.0.
3. Wash.
4. Oxidize with 1% (w/v) aqueous periodic acid for 2 h at room temperature.
5. Wash in running water for 10 min.
6. 0.5% (w/v) aqueous phenylhydrazine hydrochloride for 2 h at room temperature.
7. Wash in running water for 5 min.
8. Incubate in distilled water for 16 h at room temperature.
9. Stain with freshly prepared Basic Fuchsin Schiff for 4 h.

Appendix 6

Table A6.1. Apical Minor Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	9.38±0.59[1] (12)[2]	6.90±0.44 (6)	7.25±0.48 (6)	8.59±1.12 (6)	10.46±0.50 (6)	8.47±0.53 (6)	6.94±0.36 (6)	11.37±0.54 (7)	8.06±0.20 (6)	7.09±0.31 (6)	10.23±0.38 (7)	7.66±0.69 (5)	6.92±0.62 (6)
Transverse	10.50±0.38 (12)	11.20±0.90 (6)	7.56±0.40 (6)	14.07±1.22 (6)	15.48±0.94 (6)	8.65±0.23 (6)	10.16±0.46 (6)	14.93±1.02 (8)	8.95±0.58 (6)	9.68±0.55 (6)	14.30±0.56 (8)	8.78±0.35 (6)	10.31±0.63 (6)
Descending	9.76±0.57 (12)	16.44±1.16 (6)	8.13±0.27 (6)	16.31±1.05 (6)	23.08±1.92 (6)	9.87±0.38 (7)	14.20±1.24 (7)	18.97±1.71 (7)	11.67±1.53 (6)	12.79±1.06 (6)	14.13±0.62 (6)	10.08±0.78 (6)	11.64±1.29 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		7.88±0.71 (6)	5.86±0.17 (6)	7.17±1.15 (5)	9.76±0.51 (6)	7.17±0.29 (6)	7.45±0.31 (6)	8.81±0.33 (7)	7.02±0.56 (6)	5.65±0.30 (6)	9.86±0.58 (6)	6.29±0.25 (6)	7.85±0.46 (6)
		10.12±0.98 (6)	8.61±0.38 (6)	10.30±0.73 (5)	11.73±0.49 (6)	9.53±0.35 (6)	8.61±0.58 (6)	10.42±0.42 (7)	8.42±0.13 (6)	8.47±0.42 (6)	11.26±0.24 (9)	8.41±0.34 (6)	8.50±0.44 (6)
		13.46±2.60 (6)	10.33±0.34 (6)	11.64±1.01 (5)	14.24±0.37 (6)	8.99±0.50 (6)	10.16±0.64 (6)	11.05±0.42 (6)	8.73±0.22 (6)	8.88±0.42 (7)	11.69±0.51 (9)	10.52±0.57 (6)	11.53±0.43 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	3.11	<0.001	<0.05 NC[a]

* Distance from cell surface to cell surface across lumen (micrometers).

** Following surgery.

[1] Mean ± SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[a] NC = Not calculated for Segment x Group x Week (See Table IV.2).

Table A6.2. Apical Major Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	53.2±2.6[1] (12)[2]	48.8±2.2 (6)	38.4±2.2 (6)	39.6±3.1 (6)	66.3±1.1 (6)	39.6±1.1 (6)	38.2±1.2 (6)	58.9±2.1 (7)	37.9±1.4 (6)	38.1±1.8 (6)	57.0±1.3 (7)	39.1±1.3 (5)	38.2±1.8 (6)
Transverse	51.4±2.8 (12)	44.6±2.6 (6)	37.7±1.9 (6)	50.4±3.0 (6)	59.8±3.8 (6)	38.5±3.1 (6)	42.9±1.8 (6)	59.9±3.0 (8)	42.1±0.9 (6)	42.5±2.9 (6)	56.3±2.2 (8)	43.1±2.0 (6)	46.5±2.8 (6)
Descending	55.9±2.3 (12)	61.9±3.3 (6)	45.6±3.3 (6)	56.5±1.8 (6)	79.0±4.7 (6)	52.8±2.0 (7)	62.4±2.9 (6)	67.1±3.0 (7)	66.6±5.4 (6)	68.0±1.9 (6)	61.4±2.1 (6)	53.7±2.9 (6)	59.8±3.7 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		56.2±2.2 (6)	32.3±1.0 (6)	40.5±5.4 (5)	54.5±1.6 (6)	39.2±1.4 (6)	40.1±1.3 (6)	48.9±2.6 (7)	37.9±0.4 (6)	34.8±1.1 (6)	56.2±2.0 (6)	37.8±1.7 (6)	41.0±1.7 (6)
		47.2±1.4 (6)	44.1±1.7 (6)	49.5±4.2 (5)	53.1±2.0 (6)	48.8±1.5 (6)	43.5±1.4 (6)	50.0±0.9 (7)	40.2±1.7 (6)	40.7±2.0 (6)	55.0±1.5 (9)	44.3±3.2 (6)	43.5±2.4 (6)
		56.9±3.5 (6)	58.2±3.1 (6)	51.9±1.8 (5)	57.9±1.5 (6)	54.5±2.1 (7)	52.0±1.8 (6)	54.1±3.3 (6)	50.3±1.3 (6)	53.3±1.4 (7)	62.2±2.0 (9)	56.3±3.7 (6)	59.4±2.5 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.21	<0.01	<0.05 NC[a]

* Distance from basement membrane to basement membrane (micrometers).

** Following surgery.

[1] Mean ±SD.

[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:

[a] NC = Not calculated for Segment x Group x Week (See Table IV.2).

Table A6.3. Medial Minor Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	8.88±0.58[1] (12)[2]	6.97±0.31 (6)	6.86±0.40 (6)	7.44±0.75 (6)	10.13±0.34 (6)	7.86±0.47 (6)	6.77±0.30 (6)	10.37±0.48 (7)	7.89±0.38 (6)	5.94±0.33 (6)	10.14±0.49 (7)	7.81±0.35 (5)	6.57±0.40 (6)
Transverse	8.64±0.17 (12)	8.88±0.32 (6)	7.74±0.40 (6)	12.93±0.77 (6)	13.17±0.95 (6)	7.73±0.23 (6)	9.15±0.35	12.76±1.11 (8)	7.42±0.39 (6)	8.66±0.40 (6)	13.20±0.75 (8)	8.02±0.18 (6)	9.39±0.26 (6)
Descending	8.15±0.47 (12)	13.20±0.52 (6)	7.72±0.47 (6)	15.47±1.62 (6)	14.05±0.78 (6)	8.95±0.26 (7)	12.90±1.56 (7)	13.67±0.85 (7)	10.74±1.39 (6)	10.81±0.45 (6)	11.00±0.71 (6)	9.34±0.34 (6)	9.11±0.72 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		7.44±0.65 (6)	5.68±0.09 (6)	7.06±1.04 (5)	9.79±0.63 (6)	6.73±0.28 (6)	6.87±0.34 (6)	8.77±0.37 (7)	6.38±0.33 (6)	5.34±0.32 (6)	8.85±0.34 (6)	6.24±0.27 (6)	7.19±0.32 (6)
		10.00±0.75 (6)	7.78±0.59 (6)	9.38±0.96 (5)	11.06±0.40 (6)	8.24±0.43 (6)	8.15±0.54 (6)	9.88±0.32 (7)	7.61±0.22 (6)	7.64±0.34 (6)	9.93±0.25 (9)	7.84±0.43 (6)	7.32±0.34 (6)
		11.45±1.39 (6)	8.93±0.25 (6)	9.43±0.65 (6)	10.98±0.27 (6)	7.72±0.42 (7)	9.35±0.49 (6)	9.18±0.40 (6)	8.01±0.29 (6)	8.07±0.27 (7)	9.12±0.45 (9)	10.48±1.73 (6)	9.13±0.41 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	3.25	<0.001	<0.05 NC[3]

* Distance from cell surface to cell surface across lumen (micrometers).
** Following surgery.
[1] Mean ± SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] NC = Not calculated for Segment x Group x Week (See Table IV.2).

Table A6.4. Medial Major Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	47.0±2.3[1] (12)[2]	42.6±1.8(6)	34.9±1.8 (6)	34.9±1.8 (6)	57.5±1.8 (6)	37.2±0.7 (6)	36.6±0.9 (6)	52.8±1.2 (7)	37.5±1.5 (6)	35.7±1.2 (6)	51.8±1.3 (7)	39.5±1.9 (5)	37.1±1.4 (6)
Transverse	47.5±1.9 (12)	40.2±2.1 (6)	33.5±1.6 (6)	46.4±1.8 (6)	53.2±2.3 (6)	35.2±1.0 (6)	38.7±1.1 (6)	54.8±2.8 (8)	36.2±0.5 (6)	39.2±2.0 (6)	52.7±1.7 (8)	37.2±1.6 (6)	41.6±1.8 (6)
Descending	47.2±2.1 (12)	56.5±1.6 (6)	39.4±2.9 (6)	51.0±2.2 (6)	60.5±2.1 (6)	45.4±1.8 (7)	55.6±2.6 (7)	57.4±1.9 (7)	54.0±4.4 (6)	59.9±3.7 (6)	54.5±2.0 (6)	46.1±2.8 (6)	52.2±2.9 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	46.9±2.0 (6)	34.3±3.7 (6)	36.5±2.7 (5)	50.1±2.0 (6)	38.0±1.5 (6)	37.2±1.0 (6)	46.7±2.2 (7)	36.5±1.0 (6)	33.5±1.1 (6)	49.6±1.3 (6)	35.7±1.6 (6)	38.6±1.1 (6)
	43.4±1.4 (6)	38.6±2.0 (6)	40.1±2.2 (5)	50.2±1.8 (6)	43.9±2.0 (6)	39.0±1.7 (6)	47.5±0.5 (7)	37.0±1.7 (6)	37.3±2.0 (6)	50.0±1.5 (9)	40.2±2.7 (6)	38.6±1.7 (6)
	50.2±1.1 (6)	49.8±2.3 (6)	46.5±1.2 (5)	49.6±1.0 (6)	49.0±1.9 (7)	44.9±2.2 (6)	47.8±1.9 (6)	45.1±0.5 (6)	46.6±0.9 (7)	52.3±1.5 (9)	49.2±3.1 (6)	49.8±2.4 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.65	<0.001	<0.05 NC[3]

* Distance from basement to basement membrane (micrometers).
** Following surgery.
[1] Mean ±SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[3] NC = Not calculated for Segment x Group x Week (See Table IV.2).

Table A6.5. Basal Minor Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	9.08±0.68[1] (12)[2]	7.25±0.43 (6)	6.76±0.90 (6)	6.54±0.69 (6)	10.98±0.76 (6)	7.72±0.58 (6)	6.57±0.37 (6)	11.04±0.69 (7)	7.55±0.38 (6)	7.17±0.32 (6)	10.15±0.45 (7)	7.67±0.45 (5)	7.32±0.61 (6)
Transverse	11.79±0.94 (12)	9.54±1.00 (6)	11.93±0.92 (6)	17.43±1.06 (6)	15.54±1.74 (6)	10.84±0.24 (6)	10.95±1.27 (6)	18.04±2.57 (8)	9.87±0.38 (6)	10.23±0.78 (6)	16.68±0.94 (8)	7 10.52±0.71 (6)	11.95±0.92 (6)
Descending	12.11±1.19 (12)	14.53±0.49 (6)	11.45±1.24 (6)	16.14±1.44 (6)	17.44±1.35 (6)	13.38±1.05 (7)	16.00±1.73 (7)	17.39±1.18 (7)	16.61±1.52 (6)	19.28±2.13 (6)	15.50±1.30 (6)	12.45±0.52 (6)	13.38±0.62 (6)

	Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
	7.34±0.94 (6)	6.13±0.44 (6)	7.77±1.32 (5)	9.78±0.60 (6)	8.12±0.26 (6)	6.95±0.45 (6)	9.02±0.75 (7)	6.57±0.40 (6)	5.48±0.43 (6)	8.89±0.66 (6)	5.89±0.36 (6)	8.32±0.53 (6)
	12.04±1.66 (6)	10.49±0.85 (6)	10.53±0.81 (5)	12.57±1.56 (6)	11.48±0.99 (6)	10.79±1.16 (6)	12.89±0.42 (7)	10.05±1.03 (6)	11.79±0.93 (6)	13.82±1.13 (9)	11.24±2.12 (6)	9.18±0.57 (6)
	11.05±0.68 (6)	11.99±0.43 (6)	11.51±0.31 (5)	12.29±0.53 (6)	11.71±1.08 (7)	14.08±1.66 (6)	12.99±0.57 (6)	12.38±0.81 (6)	11.38±0.64 (7)	12.74±0.58 (9)	12.81±0.65 (6)	14.37±1.17 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.49	<0.001	<0.05 NC[a]

* Distance from cell surface to cell surface across lumen (micrometers).
** Following surgery.
[1] Mean ±SD.
[2] Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[a] NC = Not calculated for Segment x Group x Week (See Table IV.2).

Table A6.6. Basal Major Crypt Width of Longitudinally Cut Glands*

Colonic Segment	Control	One Week**			Two Weeks			Three Weeks			Four Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
Ascending	43.8±1.7[1] (12)[2]	38.8±1.5 (6)	32.4±2.2 (6)	32.0±1.5 (6)	50.5±0.7 (6)	34.6±1.3 (6)	32.3±1.2 (6)	46.8±1.1 (7)	34.6±1.6 (6)	34.6±1.0 (6)	46.6±1.1 (7)	36.2±2.1 (5)	34.7±1.8 (6)
Transverse	45.5±1.9 (12)	41.4±2.1 (6)	35.1±1.0 (6)	45.5±1.5 (6)	50.8±2.9 (6)	36.0±1.1 (6)	39.1±1.9 (6)	53.2±3.3 (8)	37.2±0.9 (6)	40.0±1.9 (6)	52.6±1.6 (8)	34.4±2.2 (6)	41.8±2.5 (6)
Descending	48.2±1.7 (12)	52.7±1.1 (6)	40.4±2.4 (6)	49.4±2.5 (6)	57.4±2.3 (6)	47.7±3.1 (7)	55.9±2.7 (7)	56.3±2.2 (7)	59.5±4.8 (6)	61.7±4.4 (6)	52.5±2.0 (6)	48.6±2.2 (6)	52.1±2.3 (6)

		Six Weeks			Eight Weeks			Ten Weeks			Twelve Weeks		
		IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP	IF/CN	BP/CN	IF/BP
		41.9±1.9 (6)	28.8±1.0 (6)	35.4±3.2 (5)	44.4±1.5 (6)	37.4±0.8 (6)	35.7±1.2 (6)	41.4±1.2 (7)	34.1±1.0 (6)	30.6±1.0 (6)	44.3±0.9 (6)	33.0±1.6 (6)	36.2±1.2 (6)
		42.7±1.4 (6)	40.5±1.6 (6)	40.2±1.5 (5)	48.5±2.2 (6)	46.5±2.9 (6)	39.8±2.2 (6)	48.3±0.8 (7)	39.2±2.3 (6)	39.7±2.3 (6)	50.4±2.0 (9)	42.9±3.9 (6)	39.2±1.9 (6)
		45.7±0.8 (6)	52.4±1.6 (6)	46.8±0.8 (5)	48.5±1.5 (6)	53.5±2.3 (7)	46.6±2.4 (6)	48.8±1.2 (6)	51.0±1.2 (6)	50.7±1.2 (7)	52.6±1.6 (9)	54.2±3.5 (6)	55.1±2.3 (6)

	One-way Analysis of Variance Statistics		Scheffe's Multiple Comparison Test
	F	P	P
Segment x Group x Week	2.44	<0.001	<0.05 NC[a]

* Distance from basement membrane to basement membrane (micrometers).
** Following surgery.
[1]Mean ±SD.
[2]Number in parenthesis = Number of animals.

For Scheffe's Multiple Comparisons:
[a]NC = Not calculated for Segment x Group x Week (See Table IV.2).

Figure A7.1. Mucosal sections of the ascending colon of the rat at week 12: A, controls; B, bypass controls; C, inflamed-operated and D, inflamed controls. AB/PAS, X195.

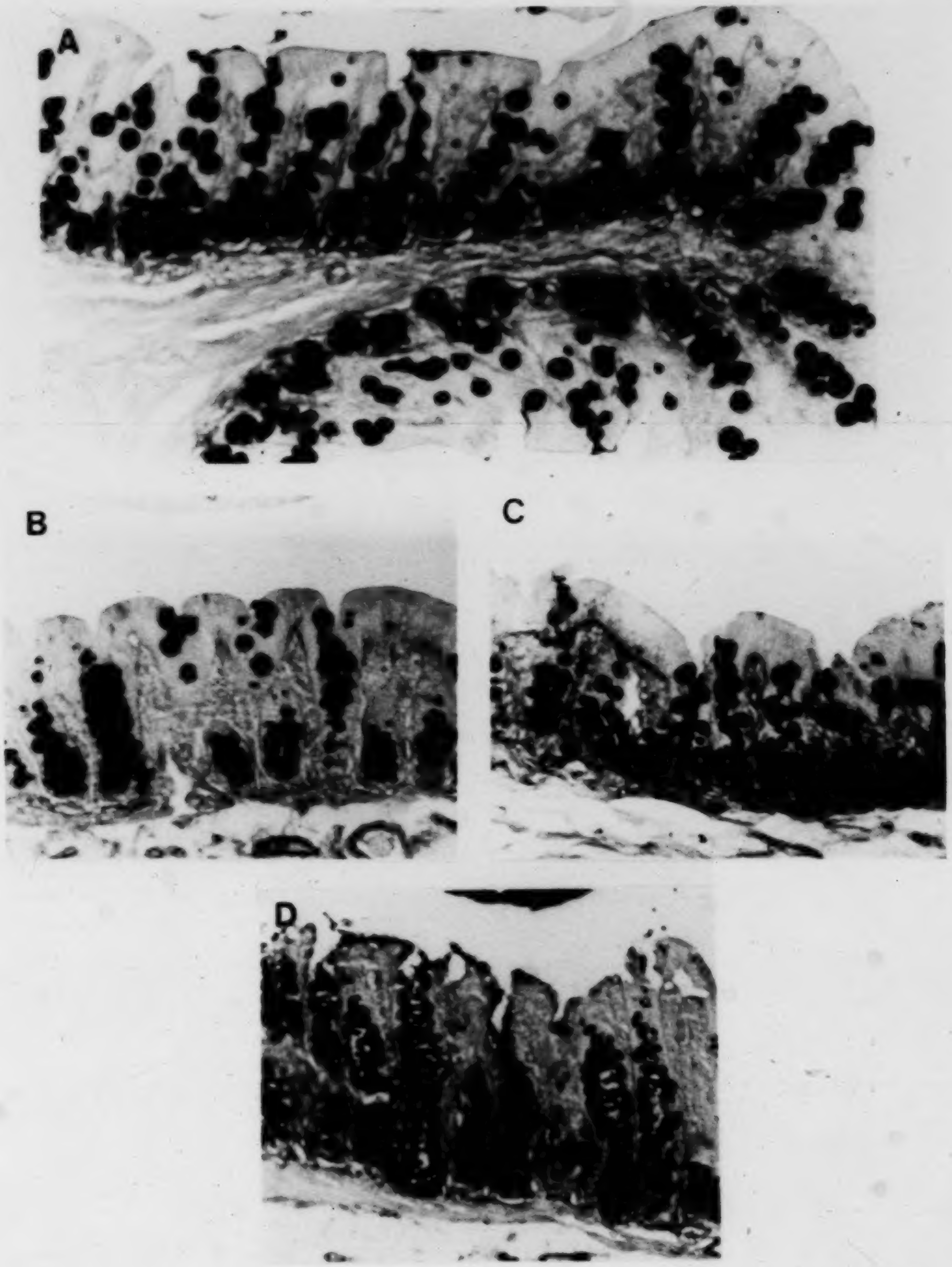

A
B
C
D

Figure A7.2. Light micrographs of the transverse colon (week 12): A, controls; B, bypass controls; C, inflamed controls; D, inflamed-operated. The reduction in mucus-containing cells is evident in the bypass sections. AB/PAS, X195.

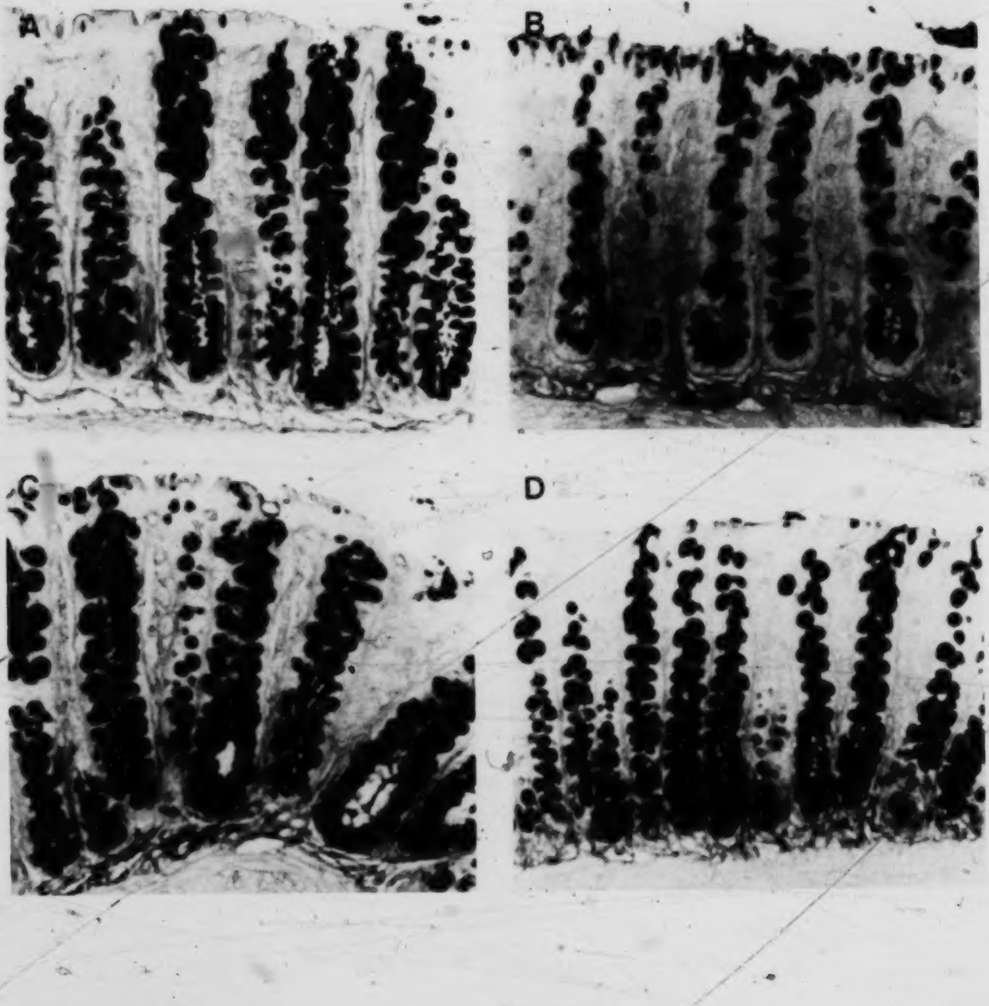

A
B
C
D

Figure A7.3. Mucosal sections of the descending colon of the rat at week 12: A, controls; B, inflamed-operated; C, inflamed controls; D, bypass controls. AB/PAS, X195.

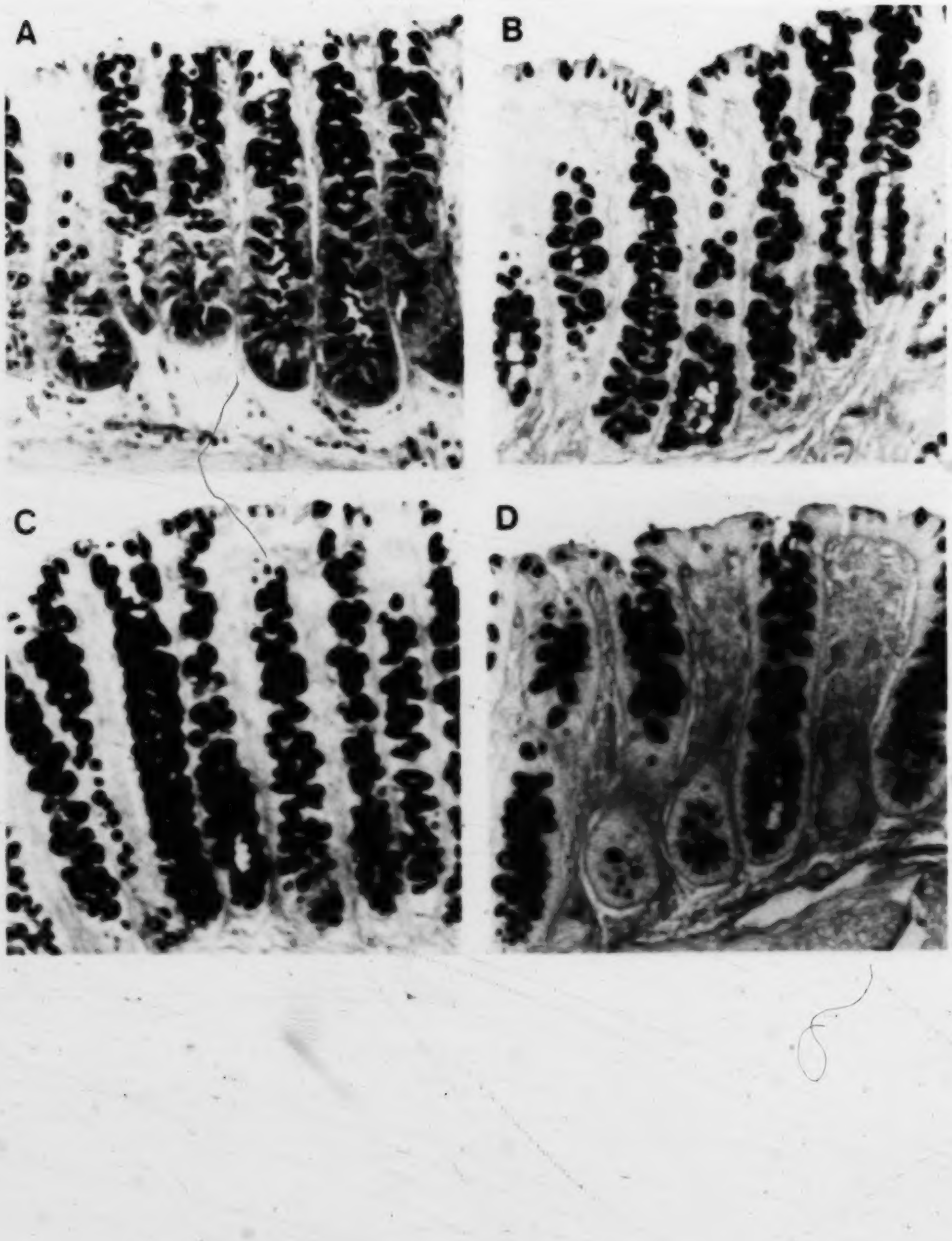

A
B
C
D

Figure A7.4A. Hyperplastic IFCN mucosa of the descending colon seen at week 1, illustrating increased inflammatory cell infiltrate. MBBF, X137.5.

Figure A7.4B. IFCN week 2 section from the corresponding region; showing ulceration at left, in addition to a hypertrophic muscularis mucosae. MBBF, X137.5.

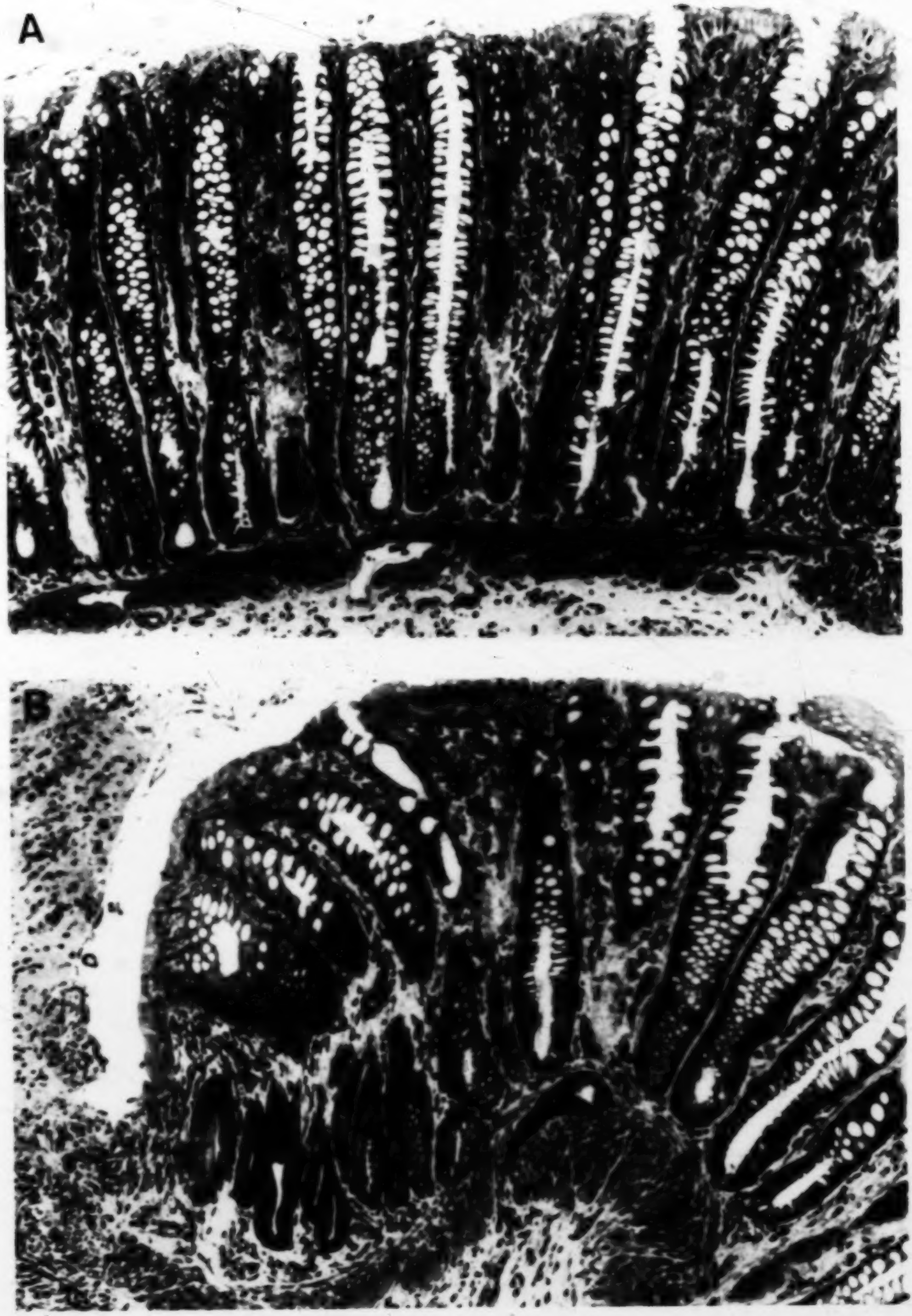

A
B

Figure A7.5. Autoradiographs of sections from the descending colon: A, control crypts; B, dysplastic-like IFCN crypts; C and D, IFBP glands at weeks 3 and 12, respectively. In B, note the numerous S-phase cells. The arrows point to the leading edge 1 hour after ^{3}H-thymidine. MBBF, X330.

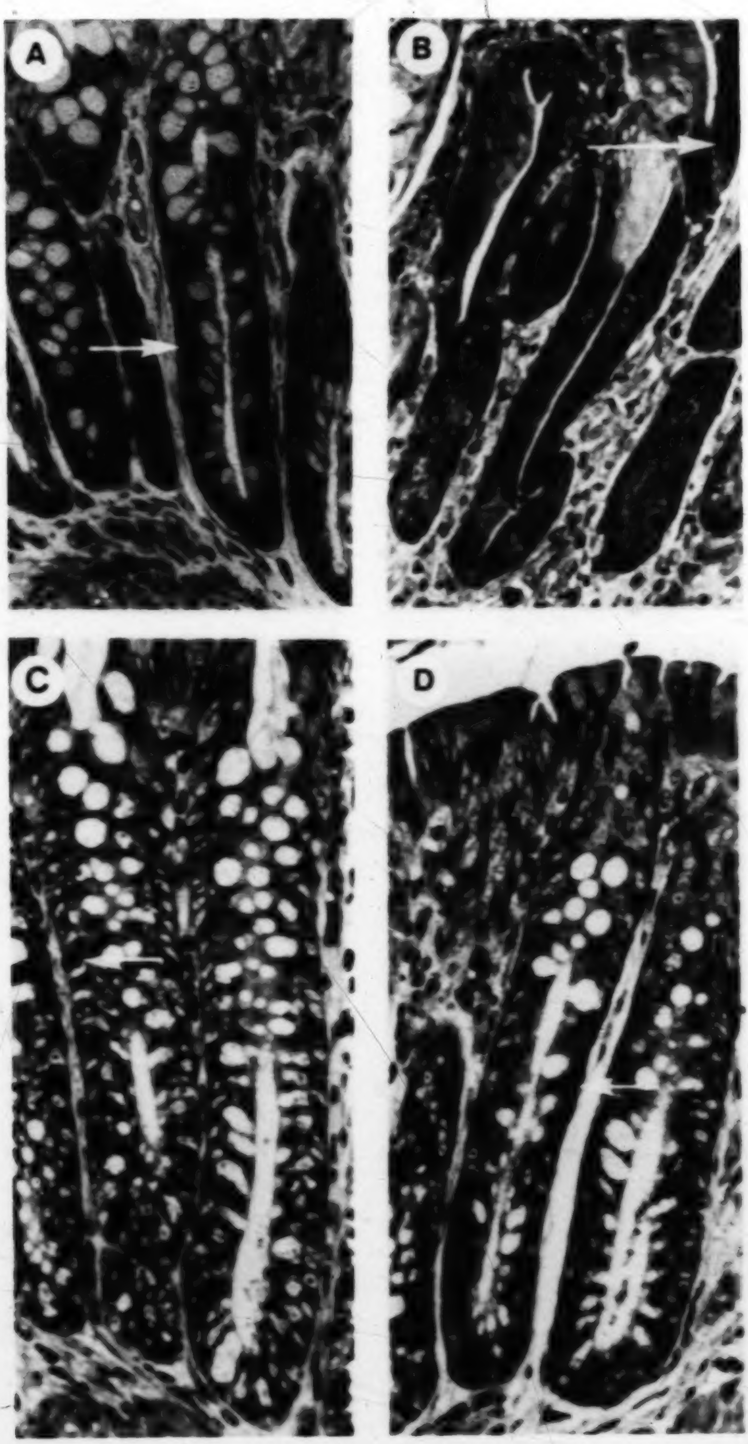
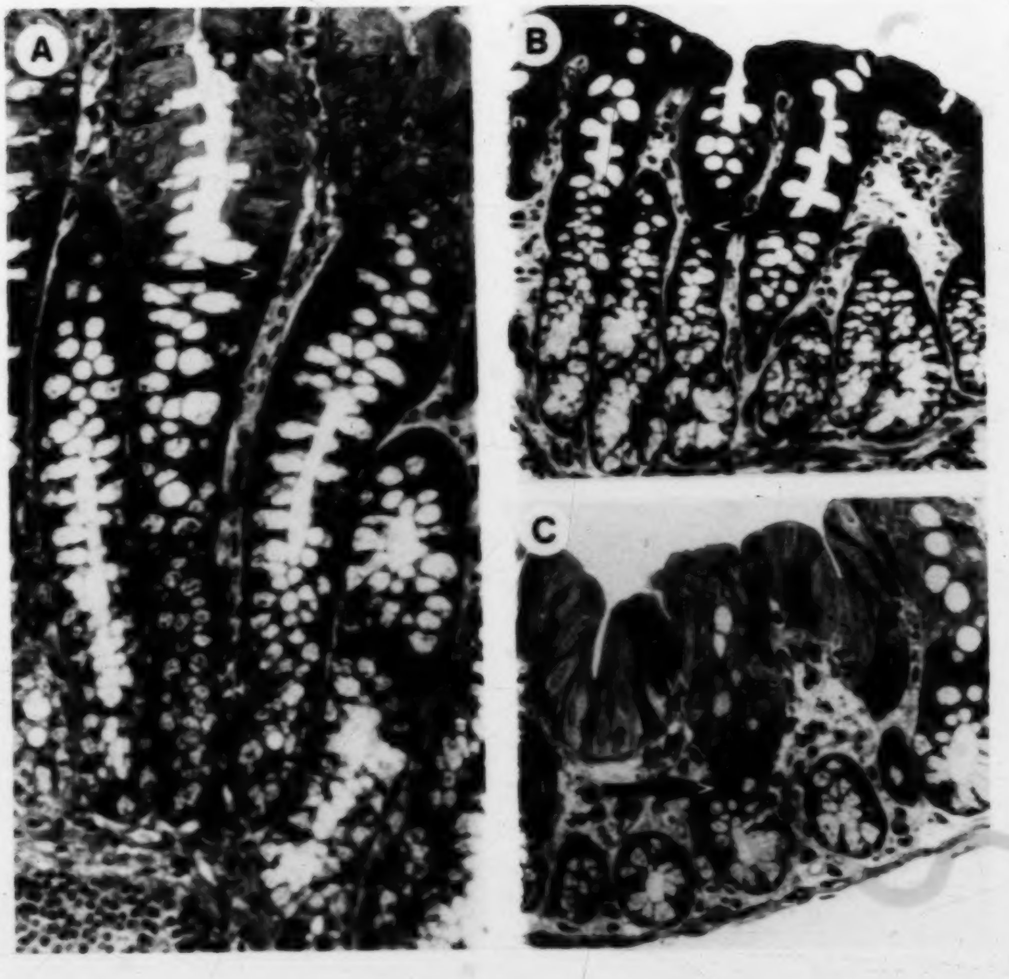

A
B
C
D

Figure A7.6. Autoradiographs of sections from the ascending colon: A, IFCN week 2 crypts (X330); B, controls (X225); C, IFBP week 8 glands (X330). Arrows point to the leading edge 1 hour after ^{3}H-thymidine. MBBF.


A
B
C